ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTES OF HEALTH

1960

NATIONAL INSTITUTE OF DENTAL RESEARCH
CLINICAL CENTER

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

US NATIONAL INSTITUTE OF DENTAL RESEARCH
Annual Narrative and Project Reports
Calendar Year 1960

CONTENTS

Narrative Reports

Administration
Information Office
Intramural
Extramural

Project Reports

OFFICE OF ASSOCIATE DIRECTOR

NIDR Project No.	Investigator(s)	Project Title
1	F. J. Kendrick	Prenatal Influences on Fetal Development.

LABORATORY OF MICROBIOLOGY

2	R. J. Fitzgerald H. V. Jordan	The Use of Germfree and Gnotobiotic Animals in the Study of Problems Relating to Oral Disease
3	E. G. Hampp R. R. Omata	Studies on Fusospirochetal Organisms and Bacteroides melaninogenicus.
4	A. Howell, Jr.	A Study of Oral Filamentous Organisms and Related Bacteria.
5	A. Howell, Jr. A. A. Rizzo	The Relationship of Pathogenic Fungi and Other Organisms to Diseases of the Oral Cavity. I. Incidence and Identity.
6	H. V. Jordan R. J. Fitzgerald	Inhibitors and Experimental Caries.
7	S. E. Mergenhagen A. A. Rizzo	Biological, Chemical, and Physical Properties of Endotoxins from Oral Bacteria.
8	T. A. Nevin	Bacteremias Following Exodontia and Periodontal Treatment.
9	R. R. Omata	Studies on the Physiology of Oral Fusobacteria and on the Proteolytic Enzymes of Microbial Origin.

RK
80
U563
1960

CONTENTS

LABORATORY OF MICROBIOLOGY (Continued)

NIDR Project No.	Investigator(s)	Project Title
10	M. Rogosa	Immunological, Biochemical, and Pathological Factors in the Taxonomy and Ecology of Oral Bacteria.
11	H. W. Scherp	Manifest and Inapparent Viral Infections of Oral Tissues.
12	T. Shiota	Nutrition and Metabolism of Lactic Acid Bacteria.
13	T. A. Nevin	Studies of Interactions Between Oral Organisms.
	LABORATORY OF BIOCHEMISTRY	
14	H. Blumenthal	Metabolism of Oral Flora and Salivary Glands.
15	M. S. Lewis	Physical Chemistry of Proteins.
16	A. H. Mehler	Enzyme Structure and Mechanism of Action.
17	K. A. Piez	Biochemistry of Amino Acids and Proteins in Mammalian Cell Cultures.
18	G. R. Martin S. E. Mergenhagen	Studies on Collagen. I. Studies on Collagenolysis. II. Studies on In Vivo Calcification.
19	E. C. Wolff	Metabolism of Amino Acids, Protein Biosynthesis, and Related Enzymology. I. Blood Clotting: Metabolism of Peptides and Enzymatic Mechanisms. II. Proteolytic Enzymes: Kinetics and Mechanism of Action.
20	J. E. Folk	Protein and Amino Acid Chemistry. I. Proteolytic Enzymes: Chemical and Molecular Studies. II. Blood Clotting: Molecular Mechanism and Enzymology.
21	R. C. Likins	Biochemical and Biophysical Studies of Calcification.
22	I. Zipkin	Biochemistry and Physiology of the Salivary Glands and Their Secretions.

CONTENTS

LABORATORY OF BIOCHEMISTRY (Continued)

NIDR Project No.	Investigator(s)	Project Title
23	I. Zipkin	The Role of Citrate in the Calcifying Tissues.
24	C. T. G. King	Correlation of Clinical Oral Health Findings to Biochemical Stress-related Factors in Personnel Wintering in Antarctica.
25	C. T. G. King	The Effect of Prenatal Factors on Fetal Development and Dental Caries in the Progeny.
26	G. R. Martin	Studies on Ascorbic Acid. I. The Transport of Ascorbic Acid. II. Role of Ascorbic Acid in Formation and Maintenance of Dental Structures.
27	F. J. McClure	The Relation of Dietary Minerals and Protein to Dental Caries.
28	R. H. Larson	Role of Bacteriology and Nutrition in Experimental Dental Caries.
29	K. A. Piez	Analytical and Structural Aspects of Collagen and Proteins of Related Interest in Relation to Function.
	LABORATORY OF HISTOLOGY AND PATHOLOGY	
30	D. B. Scott	Studies of the Structural, Physical, and Chemical Properties of Calcified Tissues by Electron Microscopy and Electron Diffraction.
31	M. U. Nylen	Studies of the Development of Calcified Tissues Under Normal and Pathologic Conditions.
32	D. B. Scott M. U. Nylen	Secondary Collaborative Projects and Training Activities.
33	J. G. Helmcke	Microscopic Studies of Special Structures in Dental Enamel.
34	P. H. Keyes	Experimental Dental Caries.
35	J. Theilade	Electron Microscopic Studies of Dental Calculus.
36	H. M. Fullmer	Histochemical Studies of Connective Tissue.

CONTENTS

LABORATORY OF HISTOLOGY AND PATHOLOGY (Continued)

NIDR Project No.	Investigator(s)	Project Title
37	M. S. Burstone	Development and Application of Histochemical Methods in the Study of Hard and Soft Tissues.

EPIDEMIOLOGY & BIOMETRY BRANCH

38	A. L. Russell C. L. White	The Epidemiology of Dental Caries - Including the Fluoride-Dental Caries Relationship.
39	A. L. Russell C. L. White	Epidemiology of Oral Diseases among Alaskan Natives.
40	N. W. Littleton	Epidemiology of Oral Diseases Among Ethiopians.
41	E. C. Leatherwood J. C. Greene	Epidemiology of Oral Diseases in Ecuador.
42	A. L. Russell E. C. Leatherwood	Epidemiology of Oral Diseases in Vietnam.
43	C. J. Donnelly	Epidemiology of Oral Diseases in Colombia, South America.
44	E. C. Leatherwood	Epidemiology of Oral Diseases in Thailand
45	C. J. Donnelly N. W. Littleton	Epidemiology of Fractured Incisors
46	A. L. Russell C. L. White	Relation Between Dental Caries and Periodontal Diseases
47	C. J. Donnelly	Study of Periodontal Disease and Caries in Adventists Families

CLINICAL INVESTIGATIONS BRANCH

48	C. J. Witkop, Jr.	Tri-racial Isolates in Eastern United States.
49	C. J. Witkop, Jr.	Hereditary Defects of Enamel and Dentin (Michigan Study).
50	C. J. Witkop, Jr.	Genetic Study in Southern Maryland.

CONTENTS

CLINICAL INVESTIGATIONS BRANCH (Continued)

NIDR Project No.	Investigator(s)	Project Title
51	C. J. Witkop, Jr.	Sickle Cell Anemias, Other Inherited Hematological Disorders, and Blood Groups.
52	J. D. Niswander	Oral Aspects of the Results of Consanguineous Marriage in Hiroshima and Nagasaki, Japan.
53	R. M. Stephan	Studies of the Oral Microbiota and Oral Environment in Relation to Dental and Systemic Diseases.
54	R. M. Stephan	Interrelationships Between Local and Systemic Factors in the Etiology of Dental Disease in Experimental Animals.
55	E. J. Driscoll	General Anesthesia (Dental) Baseline Data.
56	P. J. Coccaro	Serial Cephalometric Study of Postural Changes in Maxillary and Mandibular Full Dentures.
57	P. J. Coccaro	Serial Cephalometric Analysis of Facial Height with Natural Dentition and Following Full Denture Construction.
58	P. N. Baer	Studies on Experimental Calculus Formation.
59	P. N. Baer	Studies on Periodontal Disease in the Mouse.
60	H. R. Stanley	Histopathology of the Human Pulp.
61	R. Dubner	The Temporomandibular Joint and its Relationship to the Masticatory Musculature and the Occlusion of the Teeth.
62	R. O. Wolf A. D. Merritt	Studies in Human Saliva.
63	A. D. Merritt C. J. Witkop, Jr.	Genetics Studies in a Population Isolate (Brandywine) with Particular Reference to Hemoglobin and Haptoglobin Patterns.
64	A. D. Merritt P. N. Baer	The Oral Glucose Tolerance Test in Patients With and Without Periodontal Disease.

CONTENTS

CLINICAL INVESTIGATIONS BRANCH (Continued)

NIDR Project No.	Investigator(s)	Project Title
65	A. D. Merritt	Studies of the Urinary Excretion Products of Histidine in Patients with Liver Disease.
66	A. D. Merritt M. Kahn (NHI)	Renal Function in Patients with Sjogren's Syndrome.
67	I. I. Ship	Investigations into the Etiology of Recurrent Aphthous Ulcerations and Other Oral Non-malignant Soft Tissue Diseases.
68	I. I. Ship	Stress in the Dental Patient.
69	I. I. Ship	Secretory Sialography in Patients with Sjogren's Syndrome and Rheumatoid Arthritis.
70	I. I. Ship O. Mickelsen (NIAMD)	Effect of Dietary Phosphates on Dental Caries in Children.

NATIONAL INSTITUTE OF DENTAL RESEARCH

Annual Report of Administration

Calendar Year 1960

This portion of the 1960 narrative report relates to activities of the Office of the Institute Director which is charged with the responsibility for the over-all planning, direction, and coordination of the Institute programs.

The accomplishments, from the standpoint of both program achievement and services provided, are reflected in the reports of the extramural and intramural activities as described in the succeeding pages.

In the past year a considerable amount of program planning by the staff was devoted to the formulation of definitive plans for the orderly transition of activities to the new dental building. This move provides the Institute with its first real opportunity to expand the intramural research program. The staff has spent many hours in formulating plans involved in allocation of space, purchase of equipment, staffing, and the allocation of operating funds to the functions that will occupy this new facility. Such an undertaking not only necessitated a careful evaluation of the current programs with the idea of efficient utilization of manpower and equipment, but provided an opportunity to broaden our scope by initiating new research studies.

In connection with occupancy of the new dental facility it was necessary to revise the organizational structure to coincide with the over-all program expansion. This revision established two new sections in our organizational pattern during the year:

(1) A Section on Enzyme Chemistry in our Laboratory of Biochemistry. Grouping this category of research will greatly advance our present activities in connection with basic problems on enzymes and the kinetics of their action, and in addition will afford an opportunity for the research conducted in this section to be integrated with other basic studies pertinent to collagen and calcification.

(2) The Research Services Section, under the general direction of the Associate Director in Charge of Research, will provide better and more efficient utilization of the increased ancillary services and supporting personnel when the Institute assumes full responsibility for its own glassware and cage washing operations. In addition, this section will be responsible for animal care, diet kitchen and the maintenance of an effective safety and sanitation program.

In addition to the two new sections mentioned above, we are now in the process of establishing in our Clinical Investigations Branch, another section entitled Oral Pharyngeal Development and Function. The staff of this section will engage in studies of the normal anatomy and physiological actions of the mouth, pharynx region, and with distortions of form and functions resulting clinically from anomaly or neurological disorders.

In connection with the new dental building, the administrative staff in 1960 served as a focal point for the over-all matters relative to the occupancy of the new facility. Considerable attention was given to the task of coordinating the ordering of movable equipment, furniture, allocation of space, and staffing.

A continuous type of liaison was established with the staff of the Division of Biologic Standards and the Plant Engineering Branch to obtain the benefit of their experience with construction of Building No. 29, which is similar to ours. As a result, certain construction changes were adopted to provide a more workable arrangement of space and facilities.

Personnel occupancy of the new building in the Spring of 1961 will permit almost a 50% increase in the professional staff with a moderate increase in supporting personnel. Therefore, the planning and active recruitment of outstanding researchers began during the latter part of this reporting period. A personnel expansion of this magnitude has and will continue to have a marked impact on the staff charged with the responsibility of bringing this personnel on duty.

Worthy of mention is a significant directive issued this year by the House Appropriations Committee. This document called for separate budget submissions in 1962 for dental activities in the Public Health Service. In the past, the Division of Dental Resources, the Division of Dental Public Health, and the National Institute of Dental Research submitted a single budget request prepared by the NIDR Budget Officer. In the future, the activities of the National Institute of Dental Research will be carried as a separate appropriation item and separately defended. This action will eliminate some of the problems formerly encountered in coordinating budget preparation for three organizations in separate Bureaus.

Our future objectives will be directed toward a critical review of our total administrative program with the view of providing the organizational structure and internal procedures to meet the needs created by the program expansion.

THE NATIONAL INSTITUTE OF DENTAL RESEARCH

Annual Report on Informational Activities

Calendar Year 1960

The Dental Institute Information Office carries out a program of professional and public information that focuses on the improvement of general health through the conduct of research on oral and related diseases. This is a continuing operation with its general function specified in the Dental Research Act.

Along with the uniform expansion of informational activities in 1960, responsibilities of the Office were also broadened to include a more extensive participation in program planning and related matters pertinent to the administration of the Institute's functional program. For example, in the area of internal communication administrative responsibility was shared in the interpretation of Institute programs for members of the Executive and Legislative Branch of the Government as well as the DHEW and other agencies. Also, new programs initiated during the year polarized around the development of more effective means of communication between those organizational groups, and individuals outside the Government upon whose interest and support the success of the NIDR functional program depends in part.

PUBLIC AND PROFESSIONAL RELATIONS

Noteworthy among accomplishments in 1960 were negotiations with the Audio Visual Section of the Communicable Disease Center for the production of a motion picture (sound, color, 20 minutes) to depict activities of the National Institute of Dental Research. This orientation film, scheduled for completion in 1961, will add new dimensions to the public and professional relations program of the Institute that now extends beyond NIH to dental and other professional schools, state dental societies, the American Dental Association, state and local health departments, and the general public. With almost 800 visitors, NIDR ranked among the highest in total number of visitors received at NIH in 1960. Thus, the availability of this professionally executed motion picture will add measurably to the capacity of the Special Events Section and the Institute to more fully brief visitors on all aspects of the dental research program.

SPECIAL STUDIES

Dentistry now stands on the threshold of a "publication explosion," triggered in large part by the spontaneous Federal support of dental research and training that increased from $521,000 in 1956 to $1,700,000 in 1957. Moreover, ninety-one per cent of the total extramural funds available for research since 1950, have been appropriated during the past five years, and many grantees are now rapidly approaching the point of first publication on their project studies. This contention drew recent

support from a series of publication studies conducted in 1960 by the NIDR Information Staff.

One study consisted of an analysis of the number of grant supported papers presented at the 1957-1959 annual meetings of the International Association for Dental Research. (This is the principal meeting at which dental research is reported.) It was found, for example, that at the 1957 meeting 17 per cent of the papers presented were supported by dental grants. By 1958 this number had doubled to 34 per cent. In 1959 a one hundred per cent increase again occurred when 69 per cent of the papers presented were grant supported.

In another more extensive study, analysis was made of published papers generated by the NIDR Research Grant Program from its inception in 1950 through calendar year 1959. Data derived from available records showed the total number of published papers to be 730, abstracts not included. Further it was determined that of the 383 different investigators supported during the ten-year period, 124 had published, or roughly 34 per cent. Of the 619 different research projects supported during the same period, 174 produced publications, or roughly 28 per cent.

In a third study an analysis was made of manuscripts published by NIDR (intramural) scientists during the first ten months of last year. Editorial records showed that 71 papers were published in 31 different journals representing almost all scientific disciplines and specialities. These data, in illustrating the broad axis of dental research today, also emphasize the great difficulty of one acquiring or assimilating even a fraction of the scientific literature that now promises to inundate at least some of the dental journals.

These and other related studies are providing heretofore unavailable information to NIDR program chiefs and national advisory bodies whose actions and recommendations today are the templates of research needs in dentistry.

PROGRAM REPORTS

Internal reports prepared in whole or in part by the Dental Information Office constituted another important area of activity in 1960. Such documents, required by all Institutes, include weekly reports of selected dental research advances and program developments prepared for the Director, NIH, the Surgeon General, Congressional personnel and others; the annual report of the Dental Institute for publication in the overall annual report of the DHEW; and, an annual report on activities of the Dental Information Office. Special material for budgetary and appropriation hearings were also prepared. Such documents included the Director's Opening Statements; a compilation of highlights of research progress and programs, developments and a summary report on recent progress on oral disease for the House AppropriationsCommittee, etc. Also included among internal documents prepared or contributed to during the year were speeches for key personnel at various echelons of Government.

PUBLIC AND PROFESSIONAL INFORMATION

In concert with recommendations of the National Advisory Dental Research Council, the NIDR Information Staff has continued throughout 1960 to develop desirable outlets and design appropriate documents to better communicate research findings to dental teachers, practitioners and the general public. Two examples of such effort -- one for the professional media and one for the mass media -- are seen in the special article prepared by the Information Office for early publication in the Journal of the American Dental Association, and a feature column on NIDR research that appeared recently in Newsweek magazine. Generally, the past year saw a more than 50 per cent increase in the coverage of dental intramural research by all facets of the professional and lay media -- the result of a programed effort on the part of the Information Staff in the areas of feature articles, press, special announcements, speeches, orientations, exhibits and radio. Particular attention was directed to servicing science writers, and staff members of national publications, and house organs.

PUBLIC AND PROFESSIONAL INQUIRIES

This is a continuing function, with many requests received for information from the general public and individuals in dental and related health professions. In addition to mail, telephone, and in-person inquiries received directly by the Information Office, inquiries were also referred for appropriate handling from other areas of the Institute, the Service, the Department, other Government agencies, and members of Congress.

EDITORIAL CLEARANCE

An extensive study of the editorial clearance procedure for NIDR scientific papers was recently completed by the Information Staff. Reporting on the study, it was recommended that a new system be substituted, placing the over-all responsibility for coordination of internal clearance within the Information Office. Accordingly, this system became operative in April of last year.

DEDICATION CEREMONY

And finally, the Information Office has been assigned responsibility to coordinate the ceremonies for dedication of the new dental building in May 1961. Detailed planning for this occasion began in 1960; and, with the approach of spring, momentum on the part of the entire staff of the Information Office and the Institute is expected to accelerate sharply.

THE NATIONAL INSTITUTE OF DENTAL RESEARCH

Annual Report on Intramural Research

Calendar Year 1960

Introduction

In the relatively brief span of twelve years since the establishment of the National Institute of Dental Research, the unfolding pattern of program activities has been characterized, perhaps most significantly, by a redefinition of dentistry's scope of research.

With early emphasis directed toward the development, on a foundation of existing scientific knowledge, of a rational basis for understanding the natural history of the teeth and their supporting structures in health and disease, there rapidly evolved an era of unprecedented productivity. Today's assets may be measured, in part, by the removal of much of the artificial but traditionally structured separation of dental research from the total body of the biological and medical sciences. With this accomplishment has come new breadth, new responsibility, and new meaning to dental research. How better to herald this new era of understanding than note, without a raising of eyebrows, the essentially unchanged direction and significance of a scientist's research program as he moves organizationally to or from one of the other categorical Institutes of the NIH.

Although an expanding horizon of dental research implies an increasing availability of well-trained young scientists, the unfortunate fact is that in spite of increased college enrollments the total number of baccalaureate graduates earning Ph.D. degrees has declined in recent years. Further manpower shortages are apparent in graduate programs where the M.D. or D.D.S. degree is a prerequisite for advanced training, pathology being a notable example. Nevertheless, the evolution of graduate education in dentistry has been both rapid and gratifying, and the role of the intramural program of the National Institute of Dental Research in stimulating its further progress is significant. For example, during the past year, in the area of biophysics alone, two associates in the NIH Visiting Program and six guest workers participated in research activities which provided valuable experience in methods of electron microscopy, electron and X-ray diffraction and microradiography. From the Institute's own permanent staff, two young investigators with exceptional promise were singled out for out-of-service graduate training in the fields of human genetics and oral surgery.

While some concern over recruitment for dental research has been related to the rather widespread adoption of the basic biological sciences by graduate medical education, this acquisition probably

represents an organizational identification with schools of medicine and does not reflect the increasing association of science departments with schools of dentistry and, more importantly, with general universities. It is entirely probable that this transition is contributing to a greater flexibility of the graduate student's orientation toward a variety of the health professions, and promises increasing equality of opportunity for the advancement of broader fronts of scientific knowledge in the years ahead.

An indication of the extended horizons and new meaning of dental research is the recent establishment of two important program activities in the Dental Institute. Since enzymology has such obvious implications for all biological processes, the point is perhaps excessively belabored that a knowledge of enzymes is fundamental for an understanding of biological phenomena. Recent technical advances, however, have made it possible to study enzymes more intensively as molecules, and the prospect is thereby opened for understanding the ways in which individual enzyme activities can be altered biologically through mutations and chemically through reactions with pharmacological agents or poisons. It should be emphasized, however, that this is a vision of the future since at the present time the complete amino acid sequence is known for only one enzyme, namely, ribonuclease. But even in this case, no complete model of enzyme action has been described.

In order, therefore, to gain further insight into the essential structures and catalytic mechanisms of enzymes, a Section on Enzyme Chemistry was established in July 1960 under the able direction of Dr. Alan Mehler.

Another newly instituted program activity is concerned with the normal anatomy and physiological actions of the mouth and pharyngeal region, and with distortions of form and function resulting from anomaly or neurological disorder. Inasmuch as the major purpose of this program, to be established as a Section on Oral Pharyngeal Development and Function, is to provide a continuous interaction of studies of the normal developing human, the impaired human, and normal and experimentally impaired animals, the personnel involved will engage in various patterns of combined interests. Under the direction of Dr. James Bosma, these will include the application of available methods of pressure and sound recording, electromyography, cinematography and cinefluorography to functions of respiration, feeding and speech in subjects with orthodontic deformities, neurological impairments of the mouth and pharynx, and anomalous defects such as cleft palate and hypoplasia of the tongue, pharynx, and mandible. The very nature of these impairments dramatically emphasizes the role of the mouth and its adjacent structures in the formation of speech and in the maintenance of essential life processes of respiration and nutrition. Viewed in such logical perspective, the mouth and adjacent structures is as much a vital organ as the heart, liver or lungs.

Continuing to serve the Dental Institute in matters of long range planning and policy making, the Board of Scientific Counselors met formally on two occasions during 1960. Characteristic of its objective approach to problems was the time devoted at a recent meeting to an evaluation of Board responsibilities, and ways and means by which its mission of service might be more successfully discharged.

In taking note of those areas of research warranting increased emphasis, the Board expressed strong support for plans to (1) better coordinate and identify the diverse physical biology activities by conferring laboratory status on this important program area; (2) reorient the program of the Laboratory of Histology and Pathology toward experimental embryology with emphasis on an approach to developmental biology through methods of molecular analysis rather than along more rigid lines of classical embryology; and (3) establish an Immunology Section in the Laboratory of Microbiology in order to coordinate research in host defenses, whether mediated by specific antibodies or nonspecific mechanisms, with a view to the control of infectious diseases of the mouth and related structures; to study allergic reactions to a variety of local anesthetic agents, antibiotics and prosthetic materials used in everyday dental practice; and to facilitate the required flexibility of immunologic research programs in general.

The anticipated expansion of Institute activities made possible by occupancy of a new laboratory research building makes more urgent than ever the maintenance of an effective safety and sanitation program. Other needs related to increased animal experimentation are the coordination of animal care, special animal breeding for germfree research, and the operation of a diet kitchen and cage washing facility. Furthermore, the Dental Institute will assume, for the first time, full responsibility for its own glassware preparation facility including decontamination, cleaning, wrapping, sterilizing, special preparations, repair and delivery. In order to coordinate these many functions and assure their effective operation, request was made and authorization given to establish a central Research Services Section in the Institute.

Inasmuch as the account of laboratory and clinical research to follow is presented largely within a framework of operational categories, it should be emphasized that this mainly serves the purpose of a simplified and organized presentation and should not deprecate the more important concept of research by programs.

LABORATORY OF BIOCHEMISTRY

Collagen and Related Proteins:

In continuation studies of the chemical and structural characteristics of collagen and related proteins, recent findings by Doctors K. A. Piez and M. S. Lewis show that denaturation by heat or urea of collagen in solution results in the formation of at least two subfractions which are not identical. Although these fractions have been separated by chromatography and analyzed, it is possible that improved separation procedures may effect the resolution of still other components. Parallel crystallographic studies of protein isolated from the enamel of pig embryos, suggest the structure of a keratin although chemical analysis shows a difference in terms of a very high content of proline and a major amino acid component of histidine.

As an example of the increasing attention given to the biochemistry of amino acids and proteins in mammalian cells, collaborative studies with Dr. H. Eagle of the NIAID have shown that even though cystine is synthesized in cell culture from methionine-sulfur and glucose-carbon, it is still necessary to include it in cell culture media. This necessity stems from the observed inability of the cell to maintain its internal pool against the demands of the medium. Thus, in very large cell populations where this demand can be satisfied by more cells, cystine is no longer required. Such a seemingly simple observation assumes considerably larger proportions when it is recognized that cell culture is still a relatively new tool admirably suited for the study of many metabolic processes.

Prenatal and Dietary Factors in Experimental Dental Caries:

The experimental approach to problems of oral disease was considerably advanced this year by studying the effect of prenatal factors on the development of dental caries in the progeny. In these investigations by Dr. C.T.G. King, pregnant rats were rendered acidotic by exposure to an atmosphere of 30 percent carbon dioxide in 70 percent oxygen at different periods of gestation. It is significant that the offspring of animals treated between the seventh and ninth days of pregnancy developed a severity of dental caries (score of 19 ± 1.8) that was twice that observed in offspring of untreated control animals (score of 9.25 ± 1.23). On the other hand, acidosis after the fourteenth day of pregnancy did not alter the caries susceptibility of the offspring.

In other studies by Dr. F. J. McClure on the effect of calcium and phosphate minerals and cereal proteins on experimental rat caries,

a number of interesting findings and promising leads may be recorded. These include (1) an unexpected cariostatic effect of insoluble sodium phytate which presumably exerts its oral activity only after hydrolysis by phytase; (2) an altered mineral composition of teeth related to dietary change in calcium phosphorus ratios which, however, has no apparent influence on caries susceptibility; and (3) an increased caries activity following the feeding of certain cereals in the absence of any freely available sugar.

Approaching the oral disease problem in another dietary area, Doctors G. R. Martin and H. M. Fullmer studied the role of ascorbic acid in the formation and maintenance of dental structures. Particularly important in their examination of scorbutic guinea pigs was the observation that granules of enamel matrix accumulate in and around those ameloblasts situated adjacent to sites of defective dentinogenesis; thus suggesting that degeneration of odontoblasts in ascorbic acid deficiency is a fundamental cause of structural enamel change and a possible indirect factor in caries susceptibility.

Fluoride Studies:

The caries preventive effect of sodium fluoride administered in tablet form was reported this year in a joint publication by Doctors F. A. Arnold, Jr., F. J. McClure, and Mr. C. L. White. In this important study, the dental caries experience of 121 children who ingested 1.0 milligram of fluoride in tablet form every day for an average two-thirds of their lives was comparable to the caries score data previously reported for children of similar ages who drank water containing 1 ppm of fluoride. Although water fluoridation remains a more practical and dependable procedure for caries control, the findings in this study suggest an effective solution in those situations where fluoridation is not convenient.

The use of fluoridated drinking water by large population groups was further investigated by Dr. I. Zipkin with respect to the chemistry of human bone tissue. Over a wide range of skeletal-fluoride concentrations, the percentage of calcium and phosphorus in the bone tissues remained normal as did magnesium, sodium, potassium, and carbon dioxide. Particularly interesting, however, was the consistent slight increase of citrate which accompanied an increase in the content of fluoride in bone tissues. Additional laboratory studies have confirmed this relation of citrate and fluoride in bones of white rats.

Calcification:

The interdependence of the biochemical properties of collagen and the process of mineralization was well demonstrated by a collaborating team of investigators contributing their several specialized skills to a common but complex problem. Observing the fate of intraperitoneal

implants of purified undenatured sterile collagen gels, tendon, and decalcified dentin, it was found by Doctors S. E. Mergenhagen, G. R. Martin, A. A. Rizzo, and D. B. Scott that mineralization occurred with hydroxyapatite in crystals parallel to the axis of the collagen fibers, thus resembling normal calcified tissues. This information suggested the availability of a model system that would be subject to a variety of controllable experimental conditions, and thus useful for the study of mechanisms controlling biological calcification. As applied in subsequent investigations it was learned that a collagen gel previously dialyzed against sodium chloride solution calcifies much more slowly than that dialyzed against phosphate buffer at the same pH. Additional observations were that (1) dentin decalcified in dilute acid seems to remineralize differently from dentin decalcified in ethylenediamine tetraacetate at neutrality; (2) rat tail tendon calcifies promptly when implanted in the abdominal cavity of the donor animal; (3) collagen gel enclosed in dialysis sacs mineralizes as well as when it is freely exposed to the cellular reactions of the recipient animal; and (4) selected human oral bacteria, enclosed in dialysis tubing and implanted intraperitoneally, developed a mineral deposition morphologically indistinguishable from dental calculus.

In another study of calcification and mineral metabolism by Dr. R. C. Likins, the deposition of injected strontium^{89} and calcium^{45} was compared in several different calcified tissues of the rat. Based on X-ray diffraction line broadening analysis, it was determined that discrimination against strontium relative to calcium increased with an increase in crystal perfection. This finding of a preferential utilization of calcium over strontium in biological crystallization is in accord with previous *in vitro* observations that the final composition of a series of synthetic hydroxyapatites is related to the degree of crystal perfection, and that there exists a range of size and crystal perfection in the crystal structure of various calcified tissues including bone, dentin and enamel. In fact, this range of difference is even found to exist within adjacent regions of a single tissue such as the basal and incisal ends of a rat incisor. It is of interest to hypothesize on the discrimination in metabolism of strontium and calcium as it may relate to a means of effecting a reduction in present and future skeletal burdens of strontium^{90} due to fall out contamination of foods. Such a study is contemplated for the early future.

Enzyme Chemistry:

During the past year, Doctors J. E. Folk and E. C. Wolff, in collaboration with Dr. J. A. Gladner of NIAMD, made interesting advances in their study of the pancreatic enzyme carboxypeptidase B. A major development was the discovery of great variation in the nature of the catalytic activity with changes in the metal of the enzyme. Thus, when the originally present zinc is replaced by cadmium or cobalt, the relative activity with peptide substrates compared with ester substrates

is respectively increased or abolished. This finding provides the basis for more detailed analysis of the reaction, using the three metal ions and a variety of substrates, that may clarify our understanding of the function of metals as components of enzymes. Such studies will be greatly facilitated by the development of spectrophotometric assay methods for measuring carboxypeptidase activity with both peptide and ester substrates. These methods, using a number of synthetic substrates prepared recently, permit analyses of partial reactions to be made, thus giving additional insight into the mechanisms by which enzymes bind substrates, effect changes in the bound substrates, and release products. An unexpected finding in studies with synthetic peptides was the detection of a dipeptide (instead of an amino acid) liberated from substrates in which a basic amino acid occupied the penultimate, instead of the terminal position.

A related project, in collaboration with Doctors Gladner and K. Laki of NIAMD, has been concerned with the analysis of the proteolytic enzyme thrombin that participates in blood clotting. To date, the enzymatic capabilities of this enzyme have been tested with a number of synthetic substrates; the structures of the peptides liberated from fibrinogen studies; and complete amino acid sequences determined from both peptides, including the unusual finding of an acetyl group masking the terminal amino group of the so-called B peptide. Also under investigation is the structure of the enzyme itself.

Another project that is concerned with detailed analysis of an enzyme is the study of aldolase by Dr. Mehler. Aldolase is an essential component of glycolysis and has been known as a pure crystalline preparation for many years. During the past year the nature of this enzyme and its mechanism of action have been investigated by several technics. Direct measurement of an enzyme-substrate complex has been made using ultraviolet spectrophotometry, and the identification of this complex as the catalytically active combination has been supported by kinetic data and by experiments with inhibited enzyme. A kinetic method for assaying the reverse reaction of aldolase has been developed and used for the determination of the reaction rates and affinity for the substrates and analogues. The kinetic assays have been particularly interesting when applied to aldolase slightly modified by proteolytic enzymes, since the altered enzyme exhibits relatively different kinetic properties with various substrates.

The sum of these observations provides the basis for a theory of enzyme action, various aspects of which (e.g., the mechanism of binding of substrate to enzyme, and the role of specific groups of the protein in the catalytic process) can be tested by further experiments. Eventually it is hoped that the theoretical model will be correlated with the chemical structure. To this end, both chemical and enzymatic methods are being applied to the analysis of the primary structure of aldolase. An aspect of substrate binding that has both theoretical and technical value is the protection of aldolase against certain proteolytic enzymes by substrates and analogues of substrates. The structural requirements for this protective action are being de-

termined and are interpreted in terms of the nature of binding and the resultant modification of enzyme structure. The limited proteolysis obtained with one enzyme attacking substrate-protected aldolase yields a peptide and an altered enzyme that are currently being analyzed as part of the study of the primary structure.

One problem of enzymology is to define the metabolic pathways of individual organs and organisms in terms of the enzymes that participate in the overall reactions. Dr. H. Blumenthal has been exploring the phosphatases and diaminases of salivary glands from this point of view, and in collaboration with Dr. T. Shiota has been studying the degradation of galactosamine by oral flora.

LABORATORY OF MICROBIOLOGY

Experimental Infections:

Studies on the pathogenesis of experimental infections with oral microorganisms related to periodontal disease were continued during 1960 by Doctors S. E. Mergenhagen, A. A. Rizzo, E. G. Hampp, H. W. Scherp, G. R. Martin, and D. B. Scott. In this productive collaboration attention was concentrated primarily on (1) the isolation, purification, chemical analysis, immunological reactivity, and physiological effects of bacterial endotoxins and (2) conditions in physiological processes such as inflammation that might contribute to the progress of periodontal disease by degrading collagen fibers of the periodontium directly or by altering them to increase their digestibility by microbial proteases.

Endotoxins of the classic glucolipid type have now been isolated for the first time in quite pure state by the two-phase phenol-water extraction of Westphal from all of the major microorganisms implicated in periodontal disease; i.e., fusobacteria, selenomonads, bacteroides, veillonellae and the three main types of oral spirochetes. They were not found, however, in oral viridans streptococci and diphtheroids. The toxicity of these substances has been demonstrated in rabbits by (1) inflammation and necrosis at the sites of intracutaneous injection of microgram quantities; (2) elicitation of a marked leucocytic response; (3) elicitation, by dual injections at intervals from 6 to 48 hours, of the dermal Shwartzman reaction of hemorrhagic necrosis and the generalized Shwartzman reaction characterized particularly by bilateral renal cortical necrosis; and (4) provocation of fever in rabbits by intravenous injection of submicrogram quantities. Similar histotoxic effects of these substances have been demonstrated by injection in the oral mucosa of rabbits. As shown by pyrogenicity, the prepared endotoxins are absorbed from these sites about 25 times as effectively as from intracutaneous sites.

In view of the very recent and rapidly growing knowledge of subtle, long-term systemic effects of bacterial endotoxins, the considerable amounts of them calculated to be present in the oral cavity (in the milligram range) might well have much broader significance than

their possible contribution to the inflammatory phases of periodontal disease. Coupled with previous studies of Dr. Scherp, which demonstrated hyaluronidase production by oral viridans streptococci, chondroitinase production by oral diphtheroids, proteolytic activity of various oral bacteria, and symbiotic collagenolysis by a mixed oral flora, the more recent studies of endotoxins complete a composite picture of an armamentarium of the oral flora quite sufficient to account for the soft tissue lesions seen in periodontal disease.

Studies by Doctors Mergenhagen and Martin of dermal inflammatory sites, induced by such means as the Arthus and Shwartzman reactions in varying degrees of severity, surprisingly failed to reveal evidence of alteration of collagen at the molecular level in the form of either collagenolytic products or changed distribution of the several soluble fractions of collagen, despite microscopic evidence of disorganization of collagenous elements. However, when collagenolysis was induced in vivo with either papain or Clostridium histolyticum collagenase, specific hydrolytic products in the form of hydroxyproline-containing peptides were readily demonstrated in the circulation. Present evidence, therefore, indicates that inflammation contributes to collagen breakdown at the supramolecular level; i.e., by disrupting the organization of the collagen molecules into fibrous structures.

In a study aiming to elucidate the mechanism of calculus formation, Doctors A. Howell, Jr., and A. A. Rizzo found by combined clinical and cultural methods that streptococci and veillonellae tend to predominate during the formation of human dental calculus, contrary to the long-held belief in the predominance of filamentous types. Of further significance is the fact that some of these elements calcify when implanted in dialysis sacs in the abdominal cavities of experimental animals. While the specificity of such calcification is still under study, this laboratory approach combined with clinical studies reported in another section, should bring light to the problem of subgingival calculus, which is generally agreed to be the predominant immediate incitant of chronic periodontitis.

Viral Studies:

Viral studies directed by Dr. Scherp have achieved a working immunologic scheme for characterizing strains of herpes simplex virus, based on comparison of the kinetics of their neutralization by homologous and heterologous antisera. By making possible quantitative comparison of strains isolated in successive recurrences in a given individual, in reaction either with his own serum taken at appropriate intervals, or with sera from other persons, or with animal antisera, this procedure should elucidate hitherto inaccessible phases of the ecology of this ubiquitous virus.

Microbial Physiology:

Since Borrelia vincentii is the predominant spirochete in acute necrotizing ulceromembranous gingivitis, an important objective has been to describe clearly its metabolic activities. In a recently completed study by Dr. T. A. Nevin on the utilization of arginine as a focal nutrilite of this organism, it was shown that the metabolic process was mediated by a dihydrolase system with the production of desmidase, ureidase, and ADP. Ornithine, citrulline, ammonia, and carbon dioxide are produced, while inorganic phosphate is fixed (in both acid-labile and acid-stable compounds) in an amount equivalent to the carbon dioxide liberated. It was shown further that B. vincentii metabolizes another important nutrilite (cysteine) to pyruvate, hydrogen sulfide, ammonia, and carbon dioxide; and that an ascitic-fluid growth factor, previously shown to be essential for B. vincentii, is a peptide containing tyrosine and a sulfur-containing amino acid. Since oral bacteria producing lactic acid are probably of crucial importance in the initiation and progression of dental caries, definition of their biosynthetic mechanisms is essential. In continuing studies, Dr. T. Shiota has now demonstrated that the synthesis of folic acid by isolated enzymes of Lactobacillus plantarum proceeds via condensation of 2-amino-4-hydroxy-6-hydroxymethyltetrahydropteridine and p-aminobenzoylglutamate to dihydrofolic acid, which is then reduced to tetrahydrofolic acid by a specific reductase with either reduced di- or triphosphopyridine nucleotide as coenzyme.

Systematic Microbiology:

In collaboration with Dr. Marion Gilmour, Eastman Dental Dispensary, Rochester, New York, Dr. A. Howell, Jr., completed a definitive appraisal of the disputed taxonomic relationships of the different oral filamentous microorganisms that previously have been designated as Leptotrichia buccalis and have long been considered critically important as a matrix for dental calculus. Based on detailed descriptions of cytology, growth cycles, and metabolic activities, a separation has been recommended into two genera and species, Leptotrichia buccalis Trevisan, which has been well described hitherto and appears to belong with the lactic-acid bacteria, and Bacterionema matruchotii nov. gen. nov. comb., of which a full account is currently in press.

In the course of a two-year assignment with the National Institute for Research in Dairying, Reading, England, Mr. M. Rogosa collaborated with Dr. M. E. Sharpe to publish an extensively revised classification schema for all species of the genus Lactobacillus, based on the authors' investigations of the biochemical reactivities and serological relationships of these species (cf. the well-known Lancefield schema for streptococci). This new schema will probably achieve official status in Bergey's Manual of Determinative Bacteriology, the standard reference in bacterial taxonomy.

Continuing his morphological and biochemical studies of fusobacteria, Dr. R. R. Omata concluded that two of the three presently accepted species, F. nucleatum and F. polymorphum, were essentially indistinguishable in cultural characteristics and biochemical activities. However, the third species, F. fusiforme, differed very distinctly in morphology, did not produce indole and hydrogen sulfide, and had a homofermentative carbohydrate metabolism with lactic acid as the chief product. In these respects it corresponded very closely to Leptotrichia buccalis Trevisan.

Germfree Animal Research and Experimental Dental Caries:

A year ago, Dr. P. H. Keyes, in collaboration with Dr. R. J. Fitzgerald, provided evidence that dental caries in hamsters is a true infection. Since that time, continuing studies have been providing additional important information on the transmission of the disease through streptococcal inoculation. This has led logically to a greater emphasis on the assay of various agents thought to possess caries inhibiting potential. Examples of the activities pursued to date are (1) the demonstration by Doctors Fitzgerald and H. V. Jordan that dental caries can be induced in the gnotobiotic rat on a caries potentiating diet by infecting them with a single strain of streptococcus isolated from dental caries in conventional rats; (2) the observation by Doctors Fitzgerald and Keyes that caries developed in the normally caries-free albino hamster when another type of streptococcus, isolated from dental caries in the golden hamster, was implanted in the oral cavity, and (3) the suggestion, in collaborative studies with Dr. Jordan, that the cariogenic streptococci belong to a group that shares characteristics of the enteric and lactic streptococci in that they produce large amounts of lactic acid from common carbohydrates but demonstrate no extracellular proteolytic activity.

Significantly, the cariogenic streptococcus from the rat is without effect on the dentition of the hamster. Likewise, some seemingly identical strains are noncariogenic in the rat. Furthermore, none of the lactobacilli, diphtheroids, actinomycetes, micrococci, fusobacteria, or other types of streptococci tested to date have been cariogenic in gnotobiotic experiments. Should these preliminary observations be confirmed by current investigations in collaboration with Dr. R. H. Larson, presently held concepts that dental caries in humans can be caused by a variety of microorganisms would need reappraisal. If human caries is also found to be caused by a single, or only a few, specific microorganisms, the problem of its eventual control will be greatly simplified. One obvious possibility, although purely conjectural, would be the development of an anticaries vaccine.

In another interesting study of anticariogenic agents, Doctors Jordan and Fitzgerald extended their findings of a year ago showing sodium metabisulfite to be an effective anticaries agent in experimental animals, with the result that a new class of potential caries-inhibitory

agents has been revealed. Some of these aldehyde-trapping compounds are now being demonstrated as effective inhibitors of caries development in experimental animals and in addition exhibit pronounced antibacterial effects in vivo and in vitro. An added advantage is that some of the more active compounds have a relatively low order of toxicity, suggesting that they would be suitable for preliminary testing in humans.

LABORATORY OF HISTOLOGY AND PATHOLOGY

Biophysical Studies of Calcified Tissues:

During the past year biophysical studies have been concerned with various aspects of the structural, physical and chemical properties of calcified tissues, their development and the calcification process itself. Utilizing electron microscopy, electron diffraction, microradiography, and X-ray diffraction and spectroscopy, Doctors D. B. Scott, M. U. Nylen, and J. G. Helmcke collected new data on the ultrastructure and size of the crystallites in mature enamel and on the configurations assumed by both the inorganic and organic constituents within the large prismatic units of the enamel. These findings suggest that the long crystal-like objects characteristic of mature enamel are in reality made up of smaller units, on the order of 300A in diameter, which have developed from separate nuclei and then fused together, usually incorporating small amounts of organic matter at the fusion points. This affords an explanation for the marked morphological variation previously reported and offers a basis for understanding the widely divergent measurements on record.

Additional studies concerned with the organization of the crystalline and fibrillar elements within the prismatic units of fully mature enamel have yielded interesting new information on the cross striations in the prisms which are evident at the optical level. Contrary to the long standing opinion that such configurations are due to differences in the relative amounts of organic and inorganic substances laid down at regular intervals along the prisms, it now appears that the optical phenomenon can be explained on the basis of variations in the arrangement and alignment of the submicroscopic crystalline elements themselves. Thus, in areas which optically appear banded, the ultrastructural components seem to vary only in arrangement and not in quantity or character.

Pathogenesis of Dental Defects:

With the increasing knowledge of odontogenesis, it seemed opportune during the past year to initiate exploratory investigations into the ultrastructural manifestations of pathological conditions. For example, in a study of the effect of a rachitogenic diet on tooth development, it was found by Dr. Nylen that the ultrastructural

characteristics of the odontoblasts appear quite normal, as does the early formation of dentin matrix. However, at the time in development when the first influx of mineral should appear none can be found and the band of dentin matrix increases in thickness without becoming calcified. In contrast to the normal cytological picture of the odontoblasts, the structure of the ameloblasts is markedly altered. With respect to enamel matrix formation, there is preliminary evidence that it forms at the distal ends of atypical cells, although no conclusions can yet be made regarding its degree of abnormality.

Aside from the obvious implications of these studies in dental pathology they are of considerable importance in the establishment of criteria for the electron microscopic assessment and evaluation of minute intracellular changes. This has as a particular connotation the ultimate association of structure with function. A further outcome of this program activity is the contribution made through a number of inter-laboratory and inter-Institute collaborative projects. These include studies of ectopic calcification, amorphous mineralization systems, formation and properties of collagen, demineralization systems in lower forms, and the ultrastructure of cultured cells and their components under varied conditions.

Enamel Surface Structure and Properties:

These investigations have been in progress for a number of years and are directed toward ascertaining the changes in surface structure which occur with age, and the alterations in chemical composition and properties which can be brought about through chemical treatment. This year the refinement of data on the point-to-point alterations in specific tooth surfaces with age has reached a stage at which preliminary formulation of a useful system of age determination has been possible. The first trials of this technique indicate that it will yield accurate enough results to make it of interest in anthropology and medico-legal science, as well as in research on the surface properties of teeth themselves.

The background afforded by these and related studies have made it possible to investigate further the reactions of fluoride compounds on the external tooth surface. In preceding years it was suggested that although the mechanism of action of stannous fluoride may be somewhat different from that of sodium fluoride, a marked protection against acid action is imparted to the enamel surface by aqueous solutions of the tin preparation. Currently, attention is being directed toward the interesting problem of whether or not similar protection can be effected in vitro by incorporation of stannous fluoride in a dentifrice. Evidence, to date, indicates that if the tooth is immersed in the dentifrice proper for five minutes, there is created a degree of protection against acid attack that is quite comparable to that achieved with aqueous solutions. On the other hand, if the fluoride-containing dentifrice was applied to the tooth surface by brushing, little or no protection resulted. Investigations along these lines will be continued

due to their bearing upon the general problem of the initiation of dental caries at the tooth surface and the protection that may be afforded to tooth surfaces by chemical treatment.

Histochemistry of Connective Tissues:

In the course of the past few years, histochemical studies of connective tissue by Dr. H. M. Fullmer have provided interesting new information on the chemical composition and properties of various fibrillar components. An example of particular importance is the further study of the recently discovered oxytalan fibers which indicates that their origin may be in the mucoprotein elements situated between collagen bundles. Other histochemical studies and an exploration of various tissues for the presence of these fibers support the concept that they are most likely an elastic-type tissue in an immature form.

Additional studies by Dr. Fullmer, in collaboration with Dr. L. T. Kurland of NINDB, have demonstrated for the first time that a characteristic dermal connective tissue disorder exists in a major proportion of patients afflicted with amyotrophic lateral sclerosis; and that the disorder is most likely a primary feature of the disease rather than a sequel to the neurological damage which is most characteristic.

Maternal Influences on Fetal Development:

It is only within recent years that any appreciable attention has been directed to in utero effects on mouth and tooth development. Certainly, the rather common deformities of harelip and cleft palate have presented a confusing history with respect to their etiology. In some instances they are unmistakably related to defective genetic composition, whereas in others a clearly defined environmental factor exists. Although clinical studies are most essential to elucidate the causes of harelip, cleft palate and other defects of oral development, some of the more significant advances in knowledge have been gained by the experimental method. While too early to report conclusive findings, the recently initiated studies of Dr. F. J. Kendrick and other investigators at the Dental Institute promise to add appreciably to our understanding of congenital oral anomalies. Among the factors being evaluated are blood supply to the uterus and placenta, amniotic sac compression, and loss of amniotic fluid. To date, a high incidence of palatal clefts, limb deformities and resorptions have been observed in the term fetuses of experimental rats as compared with control groups.

EPIDEMIOLOGY AND BIOMETRY BRANCH

Nutritional Factors in Oral Disease--(Prevalence and Severity Studies in Alaska, Ethiopia, Vietnam, and Ecuador):

Whereas in previous years, investigations have concentrated primarily upon the problem of dental caries, the relatively recent recognition of the widespread occurrence of periodontal diseases and their relationship to tooth loss and disability have stimulated increased attention to the need for obtaining new data. In the United States, in individuals beyond middle age, these diseases account for the loss of more teeth than does dental caries. However, few reliable epidemiological data are available on population groups in other countries. A particular emphasis has, therefore, been given during the past year to an amplification of the descriptive and determinative characteristics of these diseases.

In collaboration with the Interdepartmental Committee on Nutrition for National Defense, Dental Institute investigators (Doctors A. L. Russell, C. J. Donnelly, E. C. Leatherwood, and N. W. Littleton) have now participated in a number of surveys which may provide significant leads to the possible influence of varying levels of nutrition on oral tissues.

While analysis of findings within and between these study groups (in Alaska, Ethiopia, Peru, Ecuador, Vietnam, Chile, Colombia, and Thailand) is incomplete, preliminary reports indicate that, by United States standards, the prevalence of dental caries is generally low whereas the prevalence and severity of periodontal diseases are correspondingly high. The following summarization suggests several promising leads.

In Alaska, extreme variations in dental caries prevalence was associated with place of residence; i.e., numbers of decayed, missing, and filled permanent teeth decreased with increasing isolation. Although Eskimo males from the principal centers of population had about the same dental caries rates as observed in white males in the continental United States, those from the more remote areas had significantly less decay. Interestingly enough, both high and low levels of caries experience were found in persons subsisting on diets that were high in animal protein, moderate in fat, and low in carbohydrate. In the case of Ethiopians using a traditionally high cereal diet which is low in sugar, low in protein, and high in carbohydrates, a uniformly low level of dental caries was observed. A similar low prevalence of dental caries was found in Vietnamese who subsist on a diet that contains highly milled rice as the staple cereal. A correspondingly low dental caries rate in Ecuadorian adults could presumably be related to optimal or high intakes of fluoride.

In contrast to the relative freedom from dental caries in the observed population groups, the only areas where periodontal disease was significantly less prevalent than in the United States were remote regions of Alaska and certain parts of Ecuador inhabited by primitive Jivaro Indians. Elsewhere in Alaska, and generally in Peru, disease levels were comparable with those seen in the United States among people of low socioeconomic status. In Ethiopia, Ecuador, and Vietnam,

prevalence levels were much above any so far recorded for the continental United States. In this regard, it is of interest to note that Ethiopians with deficient or low serum ascorbic acid levels had somewhat higher levels of periodontal diseases, whereas in Alaska this relationship was not apparent. Furthermore, in the Eskimo group, the lower prevalence of periodontal diseases could be correlated with a high thiamine excretion and a low level of plasma vitamin A.

So far as analysis of data from these studies has progressed, it appears that nutritional factors exert only a minor influence on the periodontal tissues. On the other hand, the general state of hygiene in the mouth--presence and extent of calculus and debris or their absence--seems invariably related to the condition of the periodontal tissues; i.e., within-group comparisons indicating that periodontal tissues are in a better state of health in individuals whose mouths are relatively free of these deposits.

Familial Factors in Oral Disease:

In another approach to the epidemiological study of dental caries and periodontal diseases, an investigation has been in progress to evaluate the etiological significance of familial factors. In selecting population groups for study, the desirability was recognized of having a large number of family units readily available for examination under as nearly comparable environmental conditions as possible. For this purpose, the Adventist summer camps seemed ideally suited. Preliminary findings from this study, conducted on non-Adventists as well as Adventists groups, show that dental caries prevalence is consistently lower in the latter population. However, this tendency does not hold for periodontal diseases. While too early to make any definitive conclusions, current indications are that the periodontal tissues of children tend to reflect the periodontal status of their parents in both Adventists and non-Adventists groups.

CLINICAL INVESTIGATIONS BRANCH

Clinical research during the past year has been concerned chiefly with elucidating the underlying biological processes involved in diseases related to the oral cavity, with finding more successful methods for the prevention and treatment of these diseases, and with contributing to our understanding of disease processes in general. Since the development of most oral diseases is influenced by a multitude of environmental conditions as well as biological factors which involve the entire organism, the scope of these investigations has been necessarily broad.

Dental Caries:

The past year marked the initiation of a comprehensive clinical study to evaluate the effect of dibasic calcium phosphate on the dental

caries experience of children. The research leading up to this program indicated a significant cariostatic action of this phosphate as well as other more soluble phosphates. For example, in experiments on Sprague-Dawley rats (under the direction of Dr. F. J. McClure), bread prepared with a flour containing 1.0 percent of Na_2HPO_4 resulted in a reduced caries incidence averaging 51 percent and a reduced severity score averaging 83 percent. When $(NH_4)_2HPO_4$ was used, these averages increased even further to 72 and 93 percent respectively. On the other hand, $CaHPO_4$ was found to have no inhibitory effect unless it was supplemented in the bread preparation by dietary salt. This would suggest that the relatively insoluble dibasic calcium phosphate is solubilized by NaCl, thus providing a soluble, readily available, phosphate ion in the oral cavity. Through a common ion effect, which presumably could apply to the calcium ion as well as the phosphate ion, the dissolution of enamel or dentin could then be reduced and so limit the caries decalcification process.

This hypothesis is currently being evaluated by Doctors I. I. Ship, R. C. Likins, I. Zipkin, A. L. Russell, and F. J. McClure in a group of 1800 American Indian children residing in eight Bureau of Indian Affairs and Mission schools. Four of the schools are supplied with a phosphated bread (2 percent $CaHPO_4$ in the bread flour) and four with a conventional, non-treated bread. Since only the first annual examination period has been completed by the collaborating Institutes and organizations (NIDR, NIAMD, Division of Indian Health, Bureau of Indian Affairs, South Dakota State College of Agriculture and Pennsylvania State University), it is premature to suggest any evidence of effectiveness. However, it is expected that a sufficient trial period will have elapsed by the end of fiscal 1963 to draw meaningful conclusions.

In another long range study by Dr. R. M. Stephan, a number of possible contributory factors are being evaluated in selected cases of rampant dental caries. While principal consideration has been given to microbic plaque formation, intraoral pH, food retention in occlusal fissures and interproximal areas, salivary flow rates, dietary habits, drinking water, and family history, the predominating features thus far have been overgrowths of microbic plaques on caries susceptible areas, with a predominance of mixed coccal and filamentous forms; low rates of saliva secretion; and low pH levels in caries active areas.

Periodontal Disease:

Periodontal disease continues to be one of the most common causes of oral infection and tooth loss in the adult. Seeking to further evaluate preliminary clinical impressions regarding the role of nutrition and heredity, Dr. P. N. Baer conducted a laboratory study which showed a statistically significant reduction in alveolar bone loss in an experimental group of mice maintained on a high protein diet (62 percent casein). On the other hand, a control group of animals, not on a supplemented diet, showed no protective effect. Furthermore, of

the two strains of mice studied (STR/N and DBA/2JN), the former was highly susceptible to the disease and the other relatively resistant, thereby indicating a genetic influence.

Calculus Formation:

Since it is generally recognized that calculus bears an important etiologic or contributing relationship to periodontal disease, a major effort has been directed toward the application of newer methods of study to understand better its mechanisms of formation. Although enzyme systems associated with the normal calcification process have been studied, there has been little or no information published on the relationship of known enzyme systems (other than phosphatases) to pathologic calcification. In a recently completed report by Doctors Baer and M. S. Burstone, technics of enzyme histochemistry were applied, presumably for the first time, to the study of calculus formation. By fastening mylar strips to the lingual surfaces of the lower incisor teeth of a series of patients with a diagnosis of periodontitis, the collected calculus material could be removed and incubated in substrate solutions for the demonstration of esterase activity. Chosen as substrates were acetates of AS-naphthols because of the potential chromogenicity and insolubility of the enzymatically released naphthol. Following this treatment, it was noted that the bulk of the deposits on the strips (which consisted of clumps of coccoid and filamentous forms of bacteria, leukocytes, macrophages and epithelial cells) had an occasionally variable but intense esterase activity. In considering the significance of this finding, it was suggested that enzyme activity in calculus deposits is correlated with a saponification process ultimately leading to calcifications.

Stomatitis:

A viral etiology of recurrent aphthous ulcerations has been suggested by many authors, and herpes simplex virus has been mentioned most frequently. Because of the similarity of factors that can initiate recurrences of so-called "fever blisters" and "canker sores", and because of the proximity of the two lesions, there has been considerable controversy on this point. However, evidence has now been accumulated by various investigators, including several at the Dental Institute, to show that recurrent aphthous lesions are not caused by herpes simplex virus, or another virus. For example, tissue culture technics, using monkey kidney, human skin, rabbit kidney and HeLa cells have failed to isolate herpes simplex virus, or other viruses, from one-hundred aphthous ulcerations in thirty-one patients whereas herpes virus only was isolated from thirteen of twenty-five recurrent labial vesicles in eleven patients.

In a current clinical study (conducted by Dr. I. I. Ship in collaboration with investigators at the University of Pennsylvania) of approximately 2,000 students at the University of Pennsylvania, several additional findings of interest may be noted: (1) 52 percent

of the males and 57 percent of the females had a history of recurrent aphthous ulcerations; (2) 38 percent of all the students had a history of recurrent herpes labialis; (3) age, racial background, and various social and environmental differences were not noted in the two categories of disease; and (4) a statistically significant relationship existed between emotional factors and recurrent aphthous ulcerations.

Sjogren's Syndrome:

An increasing interest in this syndrome was expressed by Dental Institute investigators during the past year. Characterized, in varying degrees, by keratoconjunctivitis, xerostomia, salivary gland enlargement and rheumatoid arthritis, an effort was made to elucidate the processes responsible for the dry mouth component. In collaboration with Dr. K. J. Bloch and others in the NIAMD, over 20 patients with Sjogren's syndrome have been studied to date. Measured by use of a Lashley cup, a marked reduction or complete absence of salivary flow from the parotid gland was observed in a number of cases. When studied by secretory sialography, a pronounced sialectasis was noted in all patients. Continuing studies are being conducted in two groups; patients with Sjogren's syndrome (with rheumatoid arthritis) and patients with rheumatoid arthritis without Sjogren's syndrome. Findings to date suggest that the syndrome may be due to an autoimmune process which interferes with the function of mucous glands; i.e., in attempting to demonstrate antibodies to saline extracts of human salivary gland, there is evidence that sera of patients with Sjogren's syndrome reacted with the extract and fixed complement.

Dental Pulp:

In addition to the common route of pulpal infection, via deep penetration of caries, a condition of non-infective injury may frequently result from the heat, pressure and chemical agents related to operative dentistry procedures. Based on observations by Dr. H. R. Stanley, it is apparent that the major factors determining pulp response to operative technics are the composition of the restorative material (silicate, self-curing resin, zinc oxide and eugenol, oxyphosphate zinc cement, etc.), the rotary speed and applied pressure of instrumentation, and the thickness of dentin. Of particular significance is the finding that the new range of high rotary speeds, in addition to being surgically efficient, provides a considerable degree of protection to the pulp. Thus, 50,000 rpm, with proper water-spray coolant and carbide or diamond cutting instrument, is quite harmless to the pulp, whereas the more conventional speeds of 6,000 to 20,000 rpm are damaging in spite of cooling agents.

Anesthetic Agents:

Since general anesthesia for ambulatory dental patients differs in certain major respects from the usual procedures of hospital anesthesia, there is need to obtain baseline physiological data if we are to assure its judicious use and provide a basis for comparison between the variety of existing anesthetic agents. During the past year, Dr. E. J. Driscoll, in collaboration with Doctors G. R. Christenson and C. L. Hebert of the Anesthesiology Department, Clinical Center, assembled data to determine objectively from brain wave activity the precise anesthetic levels at which various oral surgical procedures are carried out with the barbiturates, Pentothal, Neraval and Methahexitol. Of particular interest was that supplementation with nitrous oxide and oxygen does not significantly alter the E. G. patterns, nor does short periods of apnea as previously suggested in the literature. Also, Methahexitol causes a slightly higher blood and pulse pressure than does either Pentothal or Neraval. However, despite this 10 percent elevation, the pattern of rise is quite comparable with all three drugs. Other pertinent findings were related to the evaluation of atropine, scopolamine, and hexocyclium. When given prior to barbiturate administration it was noted that: (1) atropine alone accelerated the pulse within one minute whereas hexocyclium had no such effect until the barbiturate is added; and (2) hexocyclium is a far superior drying agent to both atropine and scopolamine, as well as having a much shorter duration of action.

The type of information being assembled in these clinical studies is particularly important since without such baseline data, the constant search for safer and more effective anesthetic agents applied to oral surgery has no foundation.

Cephalometric Studies:

In a serial cephalometric study of postural changes in maxillary and mandibular full dentures, Doctors P. J. Coccaro and R. S. Lloyd reported a pronounced backward and downward change in mandibular position, and a progressive upward change in maxillary position following insertion. The related changes in morphologic facial height were greater during the first six-month period than in the six-to-twelve-month period, and were primarily noted in the middle facial region rather than in the upper or lower face. Such information, while relatively simple to obtain, demonstrates the important role that cephalometric roentgenography may play in achieving more functional full denture construction. To this end, a continuation program is planned for the collection of pre-extraction records of skeletal and soft tissue facial profiles which will provide a clinical record of morphologic facial height in balance with facial musculature and the natural dentition.

Genetic Studies:

In the years 1958 and 1959, a Child Health Survey was conducted in Hiroshima and Nagasaki, Japan under the sponsorship of the U. S. Atomic Energy Commission, the Rockefeller Foundation, the Association for Aid to Crippled Children, and the National Institute of Dental Research. Using facilities of the Atomic Bomb Casualty Commission, and with the cooperation of the Japanese National Institute of Health, a team of examiners, including geneticists, pediatricians, dentists, and anthropologists undertook to examine a large number of offspring of consanguineous marriages. Included in the total group of 5,033 children were records of many who were deceased. While too early to report any major findings, an example of the type of important information to be gained from this study is a reported death rate that is twice that occurring in a Japanese control group (preliminary study by Dr. W. J. Schull of the University of Michigan). With specific reference to oral aspects of the study, under the direction of Dr. J. D. Niswander, examinations have included the status of soft tissues, an index of malocclusions, recording of developmental failures, estimate of DMF and DEF rates, and anthropometric determinations. Although analysis for specific inbreeding effects has only just begun, a certain number of other preliminary findings are of interest. These include (1) a higher frequency of supernumerary teeth (3 percent) than is reported for Caucasians, with a significant sex difference (4 percent in males and 2 percent in females); and (2) an accelerated eruption of permanent teeth.

In further studies of a tri-racial isolate group in Southern Maryland, Doctors C. J. Witkop, A. D. Merritt, R. O. Wolf, B. L. Hanna, L. J. Schneiderman, and Mrs. H. R. Dyson have continued to assemble data to determine the prevalence, mode of inheritance, clinical manifestations, variations in expressivity, and other factors peculiar to the hereditary illnesses found. Examples of more recent findings are: (1) a frequency of dentinogenesis imperfecta and albinism which is the highest reported for any population (3 percent and 1 percent respectively); (2) a frequency of sickle cell trait (20.5 percent) which is the highest reported outside of Africa; (3) evidence that certain speech defects are genetically determined, as in ankyloglossia which is inherited as a dominant trait; (4) the suggestion that an hereditary block in thyroglobulin synthesis accounts for the 8 percent prevalence of goiter in the female members of the isolate population; and (5) the histochemical demonstration of a presumably abnormal protein in the matrix of teeth with opalescent dentin. Other studies have been concerned with certain hematological and serological abnormalities associated with abnormal hemoglobins, blood group and secretor factor distributions in various populations; and with laboratory methods for detecting genetic differences in saliva.

In the study of another selected inbred population group located in North Carolina, findings of interest reported by Dr. Witkop and C. L. Beale of the U. S. Department of Agriculture include: (1) an

estimate of fertility that is the highest for any known racial or ethnic group in the United States (ratio of children under 5 years of age per 1,000 women is 825 as compared with a U. S. average of 417); and (2) the first description of an hereditary form of benign intraepithelial dyskeratosis involving the oral mucosa and conjunctiva. The latter condition was shown to be transmitted as an autosomal dominant trait and to have a characteristic oral cytological pattern quite similar to that seen in Darier's disease. Following the observation of identical intracytoplasmic bodies in oral epithelial smears from patients who had received certain cancer chemotherapeutic agents (methotrexate and possibly fluorouracil, as well as those who had been on therapeutic doses of X-radiation, it was postulated that the common denominator in all the conditions was a disturbance in nucleic acid metabolism.

- - - - - - - - - - -

In this review of 1960, perhaps the one ingredient that best characterizes the total program effort is the flexibility with which the professional staff utilized its sense of anticipation for unexpected leads and exploited implied opportunities. While a true measure of accomplishment must necessarily await the test of time, the existing framework within which this freedom of scientific expression and pursuit of knowledge operates, is the best assurance for a future of productivity.

THE NATIONAL INSTITUTE OF DENTAL RESEARCH

Annual Report on Extramural Research

Calendar Year 1960

Research Grants Program

The Dental Institute again this year has continued to encourage and support fundamental research in the various basic sciences of primary importance to clinical research in dental medicine. During 1960 the number of active research grants totalled 372 at 101 institutions in 36 states and territories and 7 foreign countries as compared with 340 grants in 33 states and territories and 5 foreign countries last year.

Research progress achieved by the Institute grantees is an indication of the importance and development of the Institute's extramural programs. All of the 47 dental schools in the U. S. Have now participated in the Institute research grants program. Approximately 80% of the funds allocated for research projects are devoted to research support at these institutions. Fundamental studies in the field of dental research are directed to a reduction in the degree of empiricism and the establishment of a more scientific basis for solution to problems in dental medicine. The broad range of interests and responsibility assumed in the support of research grants through the extramural programs are partially indicated by the representative examples given below:

Noteworthy this year in the field of congenital anomalies was the award of a grant in support of an interdisciplinary research program for the study of the etiology, functional activity, and therapeutic management of a congenital cleft lip and cleft palate syndrome. This program, dentally oriented, involves a broad spectrum of investigations ranging from the embryology and genetics involved to an understanding of mechanisms, evaluation of treatment, and psycho-social factors. Cooperative projects will be undertaken utilizing the Department of Surgery, the Institute of Physical Medicine and Rehabilitation, and the Department of Psychology at New York University. The grant will allow emphasis on an integrated team approach to the cleft palate problem in the research and rehabilitation phases, and quite importantly it is designed to contribute to the training of investigators and practitioners in the field as well as to solve important problems in this general area.

Various factors which separately or together have had an important influence in producing changes in the care of people with cleft palates are: 1) the concept of the whole child; 2) the study and application of knowledge about growth and development; 3) the inadequacies arising out of the rigid compartmentalization developing in specialty medical practice; and 4) the use of the team approach to deal with complex dental-medical problems.

A fundamental part of the study is the cineradiographic analysis of speech and swallowing mechanisms. The grant is under the supervision of the program director who will integrate the efforts of 28 capable investigators working in a coordinated group effort attacking problems in a cooperative way.

Dental calculus and the mechanisms involved in its formation have stimulated widespread interest in the dental field because of their role in periodontal disease, and because of the similarity of calculus to kidney stones, teeth and bone. NIDR grantees at Harvard University studied microthin calculus sections using electron microscopy and electron diffraction. The micrographs obtained showed occurrence of two types of calcification, and throughout the mass were found the microorganisms typically seen in calculus. While microorganisms have previously been implicated in calculus formation, these observations graphically indicate for the first time that oral bacteria may themselves be subject to mineral deposition. Current studies in this field at Harvard are designed to explore calculus formation and deal with developmental stages of calcification phenomena, as well as histochemical analyses of the substances involved.

Tetracycline a broad-spectrum antibiotic frequently used in clinical practice is a compound of wide interest to dentistry both as a prophylactic measure in oral surgery, and because of its potential as a caries inhibitor. Grantees at New York University College of Dentistry have reported on studies designed to determine the possible effects of tetracycline on skeletal formation.

The Institute has supported long-term studies to determine the effects of community fluoridation. The city of Evanston, Illinois initiated a water fluoridation program in 1947 under the sponsorship of the Institute. Subsequent surveys of Evanston school children during a 13 year period revealed notable reductions in decayed, missing and filled teeth as compared with those of the neighboring non-fluoridated community of Oak Park, as well as with Evanston records prior to fluoridation. Results show that children of such ages as to achieve fluoridation benefits had an average of 2.75 fewer decayed teeth than those not participating. Data gathered from the study gave further credence to the beneficial effects of fluoridation reported in long-term studies by the Public Health Service in Grand Rapids, Michigan; Newburgh, New York; and Brantford, Ontario.

Moreover, during the year a new grant was approved which will support a study on fluorine: its effect on dentition when given from infancy. This grant, at Wayne State University College of Medicine, will support studies designed to provide data, not presently available, on the absorption and excretion of fluorine by infants. These long-term studies and clinical evaluations are directed to a determination of the effect of different methods of fluoride administration. Results from these investigations should provide a better understanding of the optimum use of fluorine in the prevention of tooth decay.

For many years dental scientists have been involved in studies of various local and systemic factors in an effort to discern the agents responsible for different oral conditions. Grantees at Tufts University School of Dental Medicine have reported on studies of the nervous systems as one of the contributing etiological factors in diseases of the oral cavity. More specifically, the purpose of the investigation was to determine the effects on the oral cavity of the chronic visceral stimulation of the brain stem. Data from the investigation have clearly demonstrated irritation of certain areas of the brain stem may effect basic pathologic changes in the oral cavity as well as in the gastrointestinal tract. Thus, these studies present for consideration the possible role that more subtle neurological disorders may play in the general behavior of oral disease.

Hyperplasis of the oral gingiva is a common reaction among epileptics following systemic administration of dilantin sodium. To explore the theory that compounds might accelerate the healing of gingival and other wounds through collagenization, a controlled study of the effect of this drug on the tensile strength of healing wounds has been performed by grantees at the Indiana University School of Dentistry. The studies have demonstrated that systemic administration of dilantin sodium produces a dramatic increase in tensile strength of healing wounds in rats and appears to be the same basic reaction that accounts for the undesirable side reaction of gingival hyperplasia in epileptics receiving this drug, although the mechanism is unknown.

The characteristics of an adhesive satisfactory for clinical application are so exacting that most really good bonding agents are ruled out on the basis of setting time alone. Briefly stated, the requirements for a satisfactory bonding agent include: adhesiveness to tooth structure, resistance to mouth fluids, lack of toxicity to living tissue, and a setting time practical for operating room conditions and patient comfort. NIDR grantees at the Children's Medical Center, Harvard School of Dental Medicine, after preliminary screening determined that a new plastic bonding agent, Eastman 910 adhesive, most nearly met the requirements of an adhesive for clinical application in dentistry. Both in vitro and in vivo tests were carried out with this adhesive, and the following factors were found to be important: 1) the enamel surface must be clean, etched with acid and dry; 2) contact between enamel and adherend is necessary to protect the bond from moisture. Moisture is the greatest deterrent to a solution of the problem. No satisfactory solution was found to the clinical objective of bonding to enamel without mechanical retention. Since the Council has assigned a high priority to work in the field of bonding agents, it is anticipated that considerable emphasis will be devoted to the support of work in this area in the future.

Gross observations of periodontal lesions in the rice rat have revealed the susceptibility of this species to periodontal disease. Grantees at Harvard University have completed detailed histologic studies which identify the stages of progression of periodontal disease in these animals. The data suggest that the inordinate high susceptibility to periodontal disease in the rice rat is associated with genetic factors,

and further establish the unique importance of this animal in periodontal disease research.

The role of calcification of teeth and its relation to human caries has been studied for several years at the Jewish Hospital of Brooklyn by NIDR grantees. These extensive studies involve investigations on: the composition of bones, teeth, body fluids, and diet; the interrelationship of caries susceptibility to diet by fluids and tooth composition; and the crystal dissolution in mineralized tissues involving detailed electron microscope investigation of collagen transformation.

Invertebrate organisms as material for the investigation of calcification mechanisms are being studied at Duke University and offer a variety of experimental approaches not afforded by mammalian calcification systems.

The Council, realizing the importance of research abroad agreed to use up to 2% of the research grant budget to scientists in foreign countries in support of especially worthwhile research. Consonant with this understanding, 11 foreign grants for approximately $66,000 were awarded during the calendar year.

Training Grant Program

The primary objective of the NIDR graduate training program is to increase the number of competent researchers and/or teachers in dental schools and other dental research institutions throughout the United States. The program is designed to meet the critical shortage of clinical and basic science personnel presently available to these institutions. It has been established that substantial numbers of additional personnel for research and teaching will be required in the near future. Despite the current increase in training budget, it is estimated that our training program is falling short of meeting any reasonable demand.

There are three general types of training grants designed to establish 1) relatively broad research training centers where multidisciplinary programs could be set up, 2) programs in only a single area of training because of the availability of important facilities in terms of research and personnel, 3) smaller grants to places with less research potential to enable younger faculty members to go elsewhere for special training.

Currently there are 38 active training grants supporting 152 trainees at 29 training centers. These are located in 19 states and the District of Columbia.

During the 1960 calendar year 11 new grants were activated. Six of these were for training centers and two supported conferences dealing with training. In addition four training centers received supplemental grants.

To bring the fiscal year of training grants into phase with the normal academic year of training institutions, all training grants are

being prorated and financed on a basis of adjusted project period dates corresponding to the government fiscal year. For most grants this is being accomplished in two steps, the first of which was carried out this year by adjusting the grants to a common beginning date of January 1. The second adjustment, to a beginning date of July 1, will be completed in the coming year. This includes forward financing of the next year's grant period from current fiscal year funds.

The amount of $800,000 has been allocated for a new program of Dental Student Training Grants. The purpose of these is to provide necessary financial assistance to American dental schools for establishing, as part of their undergraduate curriculums, formal programs for training dental students to work with chairside assistants. The long-range purpose is to help relieve, through a more effective utilization of all existing dental skills, the continuing decline in the supply of dental services. The grants will be based upon the experience of the pilot studies conducted at selected dental schools over the past four years by the Division of Dental Resources, together with information developed through the cooperation of the ADA Council on Dental Education, the American Association of Dental Schools, and the Public Health Service. One of the conferences previously mentioned as being supported by an NIDR training grant was held in this connection.

The success of the Program Directors' meeting held in 1958 initiated requests from training officials for a similar meeting in 1960. Such a meeting was held at Western Reserve University on July 18-19, 1960. Approximately 100 prominent figures in the fields of dental education and research, including delegates from 46 dental schools, attended. Discussions centered on fundamental principles of graduate training in basic sciences and in clinical sciences. Practical experience in administration and recruitment were also discussed.

Research Fellowship Program

The National Institute of Dental Research fellowship program has been offering four types of awards: 1) dental student part-time fellowships; 2) predoctoral fellowships; 3) postdoctoral research fellowships; and 4) special research fellowships.

The primary purpose of the research fellowship program is directed toward graduate training in the basic sciences. Special emphasis has been placed on increasing the number of trained individuals in the basic sciences related to the study of periodontal disease, cleft palate, and dental caries. The Institute has recognized the dental manpower shortage and the need of competently trained personnel and has sought to support sound research training, to encourage training in critical disciplines, and to anticipate the demand for support from the growing number of graduate students.

New Fellowship Grant Program

The increase in the fellowship budget from $650,000 in FY-1960 to

$760,000 in FY-1961 will allow $110,000 for establishing the new fellowship grant program. This will provide stipends for two new types of awards, the Special Fellowship Grant and the Career Research Professorship, and also for the broadened Senior Fellowship Grant, administration of which is now being undertaken by the Institute. Additional research allowances are to be provided for these awards from research grant funds. (This increase in appropriation is currently earmarked for the Career Professorship award but it is hoped that it may be used for all three as the need appears greatest according to approvals. This cannot be anticipated at this time.) Applications are currently being received for all three types of awards.

Regular Fellowship Program

Graduate students under this program are training in such basic sciences as anatomy, biochemistry, pathology, physiology, histology, etc., and in the clinical sciences of periodontia, pedodontia, and orthodontia.

During 1960 a total of $330,292 was awarded for 52 regular fellowships at 30 institutions located in 16 states, the District of Columbia, and four foreign countries. In comparison, last year 48 regular fellowships were awarded at a total of $319,354.

Dental Student Part-time Fellowship Program

The Division of General Medical Sciences is the administrative agency responsible for the Dental Student Part-time program, while the NIDR, Extramural Programs Branch retains responsibility for programming and budgetary needs. Last year the number of units available per dental school was increased from six to eight. This year, in addition to the eight units per dental school, an additional two units were made available to each School of Public Health. All 47 dental schools requested the maximum units for a total of $242,352 for 376 units. Of the 11 Schools of Public Health, eight were awarded two units each for a total of $10,368.

A continuing study of the student part-time fellowship program shows that a high percentage of participating students continue their research training by receiving support from the Post-Sophomore or Postdoctoral Research Fellowships or as fellows under the training grant program. It is considered to be an extremely successful and worthwhile program.

Annual Report on Administration

Calendar Year 1960

Analysis of Extramural Programs, during the year, included the coding of active and terminated projects and all new grants in a categorical system reflecting research activity by areas and disciplines. Adoption and development of the key sort punch card system has been a useful and practical adjunct to the program analysis function serving as the basis for the preparation of various reports which have been favorably received by the Council.

Progress has been made in the analysis and evaluation of training and fellowship programs. These programs are presently too young to allow a realistic evaluation of factual accomplishments; however, the indirect influence of these programs on dental research is already being felt. Effort is currently under way to accumulate follow-up data on fellows and trainees, and to determine long-range requirements for personnel trained in research methodology as related to dentistry. With such information, the dental profession should be in a more tenable position to meet future demands for dental scientists required to solve major problems in the field of oral health.

Serial No. NIDR-1
1. Office of Associate Director
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Prenatal Influences on Fetal Development

Principal Investigator: Dr. F. J. Kendrick

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):

Total: 2 1/2
Professional: 1
Other: 1 1/2

Project Description:

Objectives:

To study the effect of a variety of maternal factors on development of the rat fetus with special reference to oral structures.

Methods Employed:

1. Induction of anoxia by temporary arrest of maternal blood supply to left or right uterine horn.

2. Restriction of growth of uterine implantation sites and related constriction of fetuses by means of encapsulating rigid acrylic molds, and semi-rigid molds of hydrocolloid and synthetic rubber impression materials.

3. Release of fetus from uterus with and without puncture of amniotic membranes, and release of fetus from both uterus and fetal membranes.

4. Unilateral and bilateral maternal nephrectomy.

5. Administration of lathyritic chemicals.

Series No. NIDR-1

Part A (continued)

6. Determination of antigenic properties of fetal palatal tissue.

7. Determination of hydrostatic pressure of amniotic fluid.

Major Findings:

1. Cleft palates, limb deformities, and occasional other malformations resulted from uterine circulatory arrest of 10 to 60 minutes at 12, 13, 14, and 15 days of gestation. The incidence of fetal deaths was greatest after 60 minutes of circulatory arrest on the 13th day of gestation.

2. Restricting the increase in size of uterine implantation sites failed to produce any gross abnormalities, but a significant incidence of fetal resorptions was noted.

3. Release of fetuses from the uterus produced no gross malformations, but 74% resorbed. Release from the uterus and puncture of the amniotic sac produced 72% resorptions and 13% deformities including cleft palate, while 13% were normal. All fetuses released from both the uterus and fetal membranes resorbed.

4. Fetuses from rats with bilateral nephrectomy exhibited marked suppression of growth and failure of palate closure.

5. The lethal dosage of some lathyritic chemicals has been reached without the induction of fetal anomalies.

6. Arthus type lesions have been produced in rabbits by innoculation of fetal rat palate in saline and adjuvant.

7. The accuracy and reproducibility of manometric determinations of amniotic fluid hydrostatic pressure are still under study.

Significance to Dental Research:

These investigations are concerned with the etiology and pathogenesis of congenital anomalies of oral structures, an area which is at present poorly understood.

Proposed Course of Project:

Completion of studies in progress and extension to closely related problems of fetal development.

Part B included No

NATIONAL INSTITUTE OF DENTAL RESEARCH

Individual Project Reports
Calendar Year 1960

MICROBIOLOGY:

2. R. J. Fitzgerald and H. V. Jordan: The Use of Germfree and Gnotobiotic Animals in the Study of Problems Relating to Oral Disease.

3. E. G. Hampp and R.R. Omata: Studies on Fusospirochetal Organisms and Bacteroides melaninogenicus.

4. A. Howell, Jr.: A Study of Oral Filamentous Organisms and Related Bacteria.

5. A. Howell, Jr., and A. A. Rizzo: The Relationship of Pathogenic Fungi and Other Organisms to Diseases of the Oral Cavity. I. Incidence and Identity.

6. H.V. Jordan and R. J. Fitzgerald: Inhibitors and Experimental Caries.

7. S. E. Mergenhagen and A. A. Rizzo: Biological, Chemical, and Physical Properties of Endotoxins from Oral Bacteria.

8. T. A. Nevin: Bacteremias Following Exodontia and Periodontal Treatment.

9. R. R. Omata: Studies on the Physiology of Oral Fusobacteria and on the Proteolytic Enzymes of Microbial Origin.

10. M. Rogosa: Immunological, Biochemical, and Pathological Factors in the Taxonomy and Ecology of Oral Bacteria.

11. H. W. Scherp: Manifest and Inapparent Viral Infections of Oral Tissues.

12. T.Shiota: Nutrition and Metabolism of Lactic Acid Bacteria.

13. T. A. Nevin: Studies of Interactions Between Oral Organisms.

Serial No. NIDR-2
1. Microbiology
2. Gnotobiotic Sec.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Use of Germfree and Gnotobiotic Animals in the Study of Problems Relating to Oral Disease

Principal Investigators: Dr. R. J. Fitzgerald
Dr. H. V. Jordan

Other Investigators: Dr. P. H. Keyes
Dr. H. R. Stanley
Dr. B. E. Gustafsson (University of Lund, Sweden)

Cooperating Units: Germfree Services Section, Sanitary Engineering Branch, (DRS-84)
Lobund Institute, University of Notre Dame

Man Years (calendar year 1960):
Total: 3 1/2
Professional: 1
Other: 2 1/2

Project Description:

Objectives:

A continuation of Project NIDR-11 (1959) in which germfree and gnotobiotic animals are used to study dental caries, calculus formation and periodontal disease.

Methods Employed:

1. The general methods were outlined in NIDR-11 (1959). Germfree and gnotobiotic (purposely infected germfree animals) are subjected to different experimental conditions in order to determine the relative importance of various factors in the etiology of oral diseases.

2. Similar studies are conducted in conventional animals in which microorganisms suspected of having cariogenic potential are "labeled" by induction of resistance to antibiotic agents or traced by use of the fluorescent antibody technic.

Part A (continued)

Major Findings:

1. It was reported previously that dental caries could be induced to occur in germfree rats infected with a single strain of a rat oral streptococcus. Serological and physiological studies indicate that this is a previously undescribed species with some of the characteristics of both the enteric and lactic groups of streptococci. Several related types of streptococci have been tested in germfree rats and have been found to be unable to induce caries.

2. Following the demonstration with Dr. Paul H. Keyes (NIDR-11, 1959) that caries could be induced in a "caries immune" strain of albino hamsters by the oral inoculation of certain strains of hamster oral streptococci, similar studies were undertaken to characterize the caries-active strains serologically and physiologically.

The streptococcal strains which produce caries in the albino hamster are apparently all variants of a single type which has not previously been described. The rat and hamster cariogenic strains differ serologically but they have a number of biochemical characteristics in common including the ability to produce large amounts of lactic acid from common carbohydrate substrates. Significantly neither strain displays any extracellular proteolytic activity, thus casting some doubt on the theory that proteolysis of the organic matrix of the tooth is an essential factor in caries development.

3. A wide variety of other microorganisms have now been tested as potential cariogenic agents in the albino hamster. These include lactobacilli, diphtheroids, actinomycetes, micrococci, fusobacteria and other streptococci. In no case was there any evidence that these organisms could induce caries activity, although many, the streptococci in particular, were potent acid producers in the presence of carbohydrates. The streptococcus which causes caries in germfree rats is without effect on the dentition of the hamster.

4. Our experience with the intestinal microflora of rodents was utilized in a collaborative study under the direction of Dr. F. S. Daft of NIAMD and Dr. B.E. Gustafsson of the University of Lund, Sweden, working at NIAMD, in which the role of the intestinal flora in the synthesis of Vitamin K was demonstrated. It was found that the symptoms of Vitamin K deficiency in germfree rats could be completely reversed within 24 to 48 hours if these animals were monoinfected with either a single strain of

Serial No. NIDR-2

Part A (continued)

Escherichia coli or a micrococcus isolated from the intestinal tract of conventional rats. This represents the first unequivocal demonstration that a vitamin could be made available to the host animal by the specific activities of its intestinal microflora.

Significance to Dental Research:

These observations in experimental animals indicate that the microbial factor in dental caries may be much more specific than has been generally assumed. Thus far we have found only a single type of streptococcus which will produce caries in the hamster. In the germfree rat, though the number of strains tested has been far less (2 lactobacilli, 3 streptococci, 2 diphtheroids), the same situation obtains. If these observations are confirmed by future work, it would seem reasonable to look for a single etiological agent of caries in humans. The possibilities for eventual control of caries in humans if only a single organism, or even a few organisms, are involved will be increased immeasurably. The development of an anticaries vaccine for humans is only one of the several possibilities that would be presented.

Proposed Course of Project:

The general course of this project will continue to be along presently established lines. Special emphasis will be given to the possibility of influencing the course of experimental caries by immunization of the animals with caries-active streptococci. When the facilities of the Dental Research Building become available the project will be expanded to include additional studies on calculus formation and periodontal disease. These studies will be of a collaborative nature involving interested investigators in the Laboratory of Histology and Pathology, and Clinical Investigations Branch as well as members of the Laboratory of Microbiology.

Part B included Yes

Serial No. NIDR-2

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Fitzgerald, R. J., Jordan, H.V., and Stanley, H. R. Experimental Caries and Gingival Pathologic Changes in the Gnotobiotic Rat. J. Dental Res., 39: 923-935, 1960.

Fitzgerald, R. J., and Keyes, P. H. Demonstration of the Etiologic Role of Streptococci in Experimental Caries in the Hamster. J. Am. Dental Assn. 61: 9-19, 1960.

Fitzgerald, R. J., Hampp, E. G., and Stanley, H. R.: Studies on the Survival of Parenterally Inoculated Oral Treponemes in the Guinea Pig. Oral Surg., Oral Med. and Oral Path., 13: 883-890, 1960.

Fitzgerald, R. J., Jordan, H. V., Scott, D. B., and McCann, H. G. Dental Calculus and Antibiotics in the White Rat. Arch. Oral Biol. 2: 85-86, 1960.

Fitzgerald, R. J., and McDaniel, E. G. Dental Calculus in the Germfree Rat. Arch. Oral Biol. 2: 239-240, 1960.

Gustafsson, B. E., and Fitzgerald, R. J. Alteration in Intestinal Microbial Flora of Rats with Tail Cups to Prevent Coprophagy. Proc. Soc. Exp. Biol. Med. 104: 319-322, 1960.

Honors and Awards relating to this Project:

With Paul H. Keyes, $500.00 Annual Research Award of Chicago Dental Society, February 9, 1960, for Studies on Etiology of Caries.

Serial No. NIDR-3
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on Fusospirochetal Organisms and *Bacteroides melaninogenicus*.

Principal Investigators: Dr. E. G. Hampp
Dr. R. R. Omata

Other Investigators: Dr. S. E. Mergenhagen
Dr. A. A. Rizzo

Cooperating Units: American Dental Association

Man Years (calendar year 1960):
Total: 1 1/4
Professional: 1 1/4
Other: None

Project Description:

Objectives:

1. To study the mechanism of experimental intracutaneous infections in rabbits with various pure strains of spirochetes as related to endotoxin production by the organisms.

2. To study histologically intracutaneous lesions produced in rabbits with various combinations of the fusospirochetal organisms.

3. To investigate the production of spirochetal abscesses in the mucous membranes of the hamster cheek pouch.

4. To study the proteolytic activity of pure strains of the small oral treponemes and *Borrelia vincentii*.

5. To develop routine and easily reproducible techniques for surface cultivation of the small oral treponemes, *B. vincentii* and *Borrelia buccalis*.

6. Development of techniques for isolation and mass cultivation of *Bacteroides melaninogenicus* for use in studying endotoxin production.

Part A (continued)

Methods Employed:

1. Forty-liter amounts of media were employed on several occasions for mass cultivation of the small oral treponemes to obtain sufficient numbers of cells for further study of endotoxins produced by these organisms.

2. The histologic material on intracutaneous lesions in rabbits produced with fusobacteria alone, and in various combinations with spirochetes, was assembled and studied in detail for publication purposes.

3. Abscesses were produced in the mucous membranes of the hamster cheek pouch by submucosal injection of the small oral treponemes and B. vincentii. Dark-field microscopy and cultural examination of selected animal lesions was performed.

4. Multiple cultures of the small oral treponemes and B. vincentii were grown in a glutathione-containing veal heart broth with, and without, ascitic fluid. Similar uninoculated tubes of culture fluid were used for control purposes. At intervals of 2-21 days, cultures and uninoculated controls were removed for determination of protein values by the method of Lowry and of amino acids by the procedure of Rosen. These values in turn were related to cell counts obtained at these various intervals.

5. Surface cultivation of the various pure strains of spirochetes was attempted using a modified Huntoon's hormone medium with various agar concentrations. All cultures were incubated at 35°C in anaerobic jars.

6. Various types of media were used in an effort to isolate B. melaninogenicus. Numerous types of broth were studied for mass cultivation of the isolates. These organisms were utilized in a cooperative study on endotoxins.

Major Findings:

1. During this period, cooperative research resulted in the demonstration of endotoxins in the smaller oral treponemes, B. vincentii and B. buccalis. These findings were anticipated from the results obtained in previous studies on experimental infections in rabbits. These endotoxins may be primarily operative in the elicitation of experimental spirochetal lesions in animals.

Part A (continued)

2. The histologic material prepared from intracutaneous lesions elicited in rabbits by Fusobacterium nucleatum and Fusobacterium polymorphum showed no differences at the cellular level in the initiation, progression and final outcome of these infections. However, these lesions were more fulminating during the early hours than those produced with spirochetes. Phagocytosis of the fusobacteria by the neutrophilic leucocytes was demonstrated in all lesions. No evidence was encountered of the spread of the fusobacteria into the surrounding tissues beyond the infected area. However, this finding is seemingly contradictory to the local anachoretic effect evidenced by the spread of fusobacteria to preexisting spirochetal lesions in their vicinity. These observations have been conclusively demonstrated by dark-field microscopy and cultural studies, as well as by examination of silver-stained tissue sections of the same material. The mechanism of spread of the fusobacteria appears to be by way of the local lymphatics or blood stream and further studies are indicated. Whether the anachoresis of the fusobacteria to preexisting spirochetal lesions was local or hematogenous in origin, multiplication in situ was evidenced by the numerous large clumps of organisms encountered in the silver-stained sections and they exerted a synergistic effect. The most dramatic tissue responses were noted in lesions elicited by inoculation of either recombined pure cultures of fusobacteria and spirochetes, or of symbiotic cultures of the organisms. Multilocular abscesses were produced in both instances and they were fulminating in character as denoted by the inflammatory reaction. Both types of organisms were readily demonstrated in the silver stained sections.

3. Since Vincent's infection is primarily a disease of the oral and pharyngeal mucous membranes, it is essential to determine if lesions can be produced in such tissues with the oral spirochetes. Preliminary experiments resulted in the elicitation of abscesses in 24 hours in the cheek pouch of hamsters with pure strains of a small treponeme and B. vincentii. Cultures of 48-hour old lesions were negative for both types of spirochetes; however, overgrowth of contaminating organisms from the cheek pouch acidified the media to pH 6.3, which is not conducive to spirochetal growth.

4. The proteolytic activity during the growth of two strains each of B. vincentii and the smaller oral treponemes was successfully demonstrated for the first time. The parent strains of B. vincentii and the small treponeme and their adapted variants, grown without ascitic fluid, grew equally well in their respective media and showed significant degrees of proteolytic activity, which was greater in the case of the small oral

Part A (continued)

treponemes. The greatest degree of proteolysis by all strains coincided with the logarithmic growth phases, occurring during the second through the seventh day.

5. Surface colonies of strains of the smaller oral treponemes, T. vincentii and T. buccalis have been successfully grown on a glutathione-containing veal heart medium with an agar concentration of 3 to 4 per cent. The medium was enriched at the time of use with either canine ascitic fluid, defibrinated horse blood or rabbit blood. The results are not yet routinely reproducible on all plates.

6. Several strains of T. melaninogenicus were successfully obtained utilizing a modified Hunteon's hormone agar enriched with either defibrinated horse blood or rabbit blood. Various types of broth media were utilized in an effort to cultivate these organisms en masse. It was eventually found that either a modified Hunteon's hormone broth, or Oxoid's broth for fusobacteria were most efficacious. These media were enriched at the time of use with canine ascitic fluid, reduced glutathione, menadione, and hemin. If sufficient amounts of ascitic fluid were used, menadione and hemin could be omitted from the culture fluid.

Significance to Dental Research:

1. These studies will afford new knowledge on the possible significance of the fusospirochetal organisms in certain types of oral infections.

2. Since lesions may be produced in the mucous membranes of the hamster cheek pouch, the spirochetal organisms can produce infections in the types of tissues that are commonly involved in human cases of ulcerative gingivostomatitis.

3. Information may conceivably be gained on the sequence of events involved in the initiation and progression of ulcerative membranous lesions of the oral cavity.

4. Studies with T. melaninogenicus may produce information relative to the mechanism involved in the etiopathogenesis of periodontal disease.

Proposed Course of Project:

These studies will be continued and extended along the lines of research that have been described in an effort to determine what organisms are primarily responsible in the initiation, progression and outcome of ulcerative gingivostomatitis and periodontal disease.

Serial No. NIDR-3

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Hampp, E. G., Scott, D. B., and Wyckoff, R.W.G. Morphologic Characteristics of the Oral Fusobacteria as Revealed by the Electron Microscope. J. Bacteriol., 79: 716-728, 1960.

Fitzgerald, R. J., Hampp, E. G., and Stanley, H. R. Studies on the Survival of Parenterally Inoculated Oral Treponemes. Oral Surg., Oral Med., Oral Path. 13: 883-890, 1960.

Rogosa, M., Hampp, E. G., Nevin, T. A., Wagner, H. J. Jr., Driscoll, E. J., and Baer, P. N. Blood Sampling and Cultural Studies in the Detection of Postoperative Bacteremias. J. Am. Dental Assoc. 60: 171-180, 1960.

Omata, R. R., and Hampp, E. G. Proteolytic Activity of some Oral Spirochetes. J. Dental Res., in press.

Nevin, T. A., Hampp, E. G., and Duey, B. R. An Interaction between Borrelia vincentii and an Oral Diphtheroid. J. Bacteriol., 80: 783-786, 1960.

Honors and Awards relating to this project:

None

Serial No. NIDR-4
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: A Study of Oral Filamentous Organisms and Related Bacteria.

Principal Investigator: Dr. A. Howell, Jr.

Other Investigators: Mr. W. C. Murphy, III

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2 1/2
Professional: 1 1/2
Other: 1

Project Description:

Objectives:

To study the morphological and physiological characteristics of various types of filamentous organisms and related bacteria isolated from selected oral samples.

Methods Employed:

Standard bacteriological procedures, modified as necessary for the organisms under study, are being used. Organisms are being studied on the basis of (1) growth on, and fermentation of, 19 carbohydrates and 8 organic acids; (2) oxygen tolerance; (3) other biochemical tests; and (4) animal pathogenicity.

Major Findings:

1. In 1959 it was reported (Project NIDR-13) that a detailed study of morphological and physiological characteristics of 20 strains of filamentous organisms isolated from hamsters had shown them to be essentially aerobic; to require carbon dioxide for growth in certain media; to dissociate very readily; and to produce acid from 10 of 20 carbohydrates tested. During the past year this study has been extended by the inclusion of 13 additional strains. In addition, the ability of all 33 of these strains to utilize certain organic acids has been determined. In summary,

Part A (continued)

most strains utilized and fermented glucose, sucrose, inositol, lactose, maltose, raffinose, salicin, levulose and mannose; two-thirds fermented galactose; none utilized or fermented arabinose, rhamnose, inulin, xylose, ribose, α-methyl-L-glucoside, glycerol, mannitol, or sorbitol. All strains utilized pyruvate; 15 of 23 utilized lactate; 4 of 23 utilized gluconate; none grew on the other five organic acids tested. Rates of growth were determined in all substrates tested.

2. A study, comparable to that reported above of "diphtheroids" and other unidentified filamentous organisms isolated from oral samples of humans, has been begun, but the data accumulated to date are insufficient to warrant detailed discussion.

Significance to Dental Research:

A more complete study of the numerous filamentous organisms and related forms indigenous to the oral cavity should lead to easier identification of these bacteria and to a better understanding of their possible role in oral diseases.

Proposed Course of Project:

To continue the study of unidentified and unclassified filamentous organisms and related forms, including the diphtheroids, isolated from oral samples.

Part B included Yes

Serial No. NIDR-4

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Pine, L., Howell, A., Jr., and Watson, S. J. Studies of the Morphological, Physiological, and Biochemical Characteristics of Actinomyces bovis. J. Gen. Microbiol., 23: 403-424, 1960.

Gilmour, M., and Howell, A., Jr. The Classification of Organisms termed Leptotrichia (Leptothrix) buccalis. I. Review of the Literature and Proposed Separation into Leptotrichia buccalis Trevisan and Bacterionema matruchotii comb. nov. Bacteriol. Rev., in press.

Howell, A., Jr., and Pine, L. The Classification of organisms termed Leptotrichia (Leptothrix) buccalis. IV. Physiological and biochemical characteristics of Bacterionema matruchotii. Bacteriol. Rev., in press.

Honors and Awards relating to this project:

None

Serial No. NIDR-5
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Relationship of Pathogenic Fungi and Other Organisms to Diseases of the Oral Cavity.
I. Incidence and Identity.

Principal Investigators: Dr. A. Howell, Jr.
Dr. A. A. Rizzo

Other Investigators: Dr. P. Keyes

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2 1/4
Professional: 3/4
Other: 1 1/2

Project Description:

Objectives:

1. To study the incidence and identity of pathogenic or potentially pathogenic fungi and other organisms in the "normal" mouth.

2. To study the possible relationship of these organisms to dental caries, calculus formation, and to other diseases of the oral cavity or related areas.

This is a continuation of NIDR-14 (1959).

Methods Employed:

As in previous years, considerable emphasis has again been placed on the incidence and identity of both filamentous and yeast-like organisms in the oral cavity with major emphasis on the sequence of bacteriological changes which occur during the formation of calculus. For these purposes standard bacteriological methods for the isolation and identification of these organisms, modified for the organisms under study, have been employed. Complete bacteriological studies were made of approximately 40 oral samples from 6 patients. Specimens were taken at various intervals after

Part A (continued)

complete scaling and polishing of the areas to be subsequently sampled. Some of the samples were taken from supragingival and subgingival areas of periodontally involved teeth which had received no prior treatment.

Major Findings:

1. It was reported earlier (1959) that preliminary studies (based on 26 specimens from 4 patients) indicated that a variety of bacteria were possibly involved in the development of calculus. Further work during the past year lends support to this tentative conclusion.

In samples taken from the 2nd to 14th day after cleaning, gram-positive and gram-negative cocci (streptococci and Neisseria) were the predominant organisms, though large numbers of Veillonella, diphtheroids and gram-negative rods were usually present. Filamentous and other organisms were occasionally isolated.

In specimens taken after periods of 14 days or more, streptococci remained the predominant group of organisms. In these older specimens, filamentous forms were usually second in abundance; Neisseria, gram-negative rods, and fusiform bacilli were also plentiful. Spirochetes were found (by direct smear) in all specimens more than 2 days old.

Specimens, taken from both supragingival and subgingival areas of untreated teeth, also showed a predominance of streptococci. In these samples the average proportion of gram-negative organisms was roughly estimated to lie between 20 and 40 per cent.

2. It was reported in Project Report NIDR-14 (1959) that a pilot study, in collaboration with Dr. Paul Keyes, had been undertaken to determine the possible incidence of filamentous organisms in hamsters. During the past year this study was expanded by the inclusion of additional numbers and varieties of hamsters.

As reported in 1959 oral samples taken from certain hamsters yielded almost pure cultures of a filamentous organism tentatively identified as a species of Leptotrichia. Further studies have indicated that this organism is the predominant one in the oral cavity of cream hamsters and certain strains of golden hamsters. Whereas it is found very rarely in the albino variety. This is of particular interest since it is very difficult to

Serial No. NIDR-5

Part A (continued)

induce caries in these strains of golden hamsters and in the cream variety, whereas it is relatively easy to induce caries in albino hamsters.

Significance to Dental Research:

1. The finding that streptococci predominate in the calculus plaque at all stages of development is at variance with reports in the literature which indicate that filamentous forms predominate in plaques more than 14 days old. Since our findings are based on cultural rather than morphological methods, they are more likely to represent the true picture than the reports cited. The distribution of organisms in these periodontal specimens is useful in interpreting studies concerning what role particular organisms or their products may play in periodontal disease. Accordingly, the observation that gram-negative organisms are abundant in both supragingival and subgingival specimens is consistent with the hypothesis that endotoxins may be important in periodontal disease.

2. The abundance of filamentous organisms in caries-resistant hamsters points up an interesting inverse relationship between caries and a particular group of organisms. The actual significance of this relationship remains to be determined.

Proposed Course of Project:

1. To continue the study of the bacteriology of calculus formation and of other periodontal plaques.

2. To continue the study of the incidence of filamentous organisms in the oral cavity of patients with rampant caries.

Part B included No

Serial No. NIDR-6
1. Microbiology
2. Gnotobiotics Sec.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Inhibitors and Experimental Caries.

Principal Investigators: Dr. H. V. Jordan
Dr. R. J. Fitzgerald

Other Investigators: None

Cooperating Units: None

Man Years (Calendar Year 1960):
Total: 2 1/2
Professional: 1
Other: 1 1/2

Project Description:

Objectives:

1. To test compounds which can be expected to inhibit experimental caries in rats by inhibition of growth or metabolic activities of the oral microflora.

2. To detect nutritional and metabolic characteristics which differentiate cariogenic streptococci from the noncariogenic streptococci also found in the oral cavity. This is a continuation of NIDR-15 (1959).

Methods Employed:

The methods employed are essentially those described in the previous report (NIDR-15, 1959). The inhibitors selected for test are incorporated into a coarse-particle high-sugar diet (585) to observe their *in vivo* effect on experimental caries. Animal experiments are accompanied by *in vitro* bacteriological tests on pure cultures of caries-active streptococci from rats and hamsters.

Major Findings:

1. Sodium metabisulfite and two other carbonyl-binding compounds were compared for their effect on experimental caries. Carboxylmethoxylamine fed at 0.004 molal concentration in diet 585 for

Part A (continued)

the first week and at 0.002 molal concentration for the remaining 12 weeks of the experiment caused a reduction of 82 per cent in the caries score. Sodium metabisulfite fed at 0.016 molal concentration resulted in an 86 per cent reduction. The specific aldehyde-binding reagent, dimedone, (5,5-dimethyl 1,3-cyclohexanedione) at a level of 0.029 molal in diet 585 caused a 55 per cent reduction in the caries scores. Relative effectiveness of these compounds as caries-inhibiting agents generally paralleled their growth-inhibiting properties when tested against a variety of oral organisms.

2. Several different sulfur compounds, including sulfites and sulfates, were compared for their effects on experimental caries. The incorporation of equivalent effective concentrations of sodium sulfite, sodium metabisulfite, sodium hydrosulfite and sodium bisulfite inhibited sulcal caries by 89, 84, 80, and 74 per cent respectively. In contrast, sodium sulfate and sodium bisulfate were ineffective. The latter compound caused extensive wearing away of the occlusal molar sufaces and a loss of enamel on the lingual molar surfaces. None of the sulfites appeared to cause a loss of incisor pigment.

3. The possibility of vitamin destruction by metabisulfite has prompted studies on vitamin supplementation of diet 585 containing metabisulfite. Addition of a full vitamin supplement to both autoclaved and unautoclaved bisulfite diets had no effect on growth of the animals or on the inhibition of caries caused by metabisulfite.

A different approach to this problem is being utilized in current studies. Rats are being fed metabisulfite in the diet for varying and intermittent exposure times in an attempt to reproduce the caries inhibition with a decreased over-all exposure to the agent.

4. Strains of streptococci known to be either cariogenic or noncariogenic have been studied in an attempt to detect metabolic differences between the two. Carbon balance studies with these organisms have revealed no major differences in the fermentation of glucose by either type. All strains exhibited the homofermentation to lactic acid with traces of other end products such as carbon dioxide, formate, acetate, and acetoin also produced. The hamster and rat strains differed in the production of ethanol by the latter.

Serial No. NIDR-6

Part A (continued)

Significance to Dental Research:

The studies described in this report constitute a rational approach to the discovery of chemical agents which may have potential value in the problem of caries control. The compounds under study have a known chemical effect, i.e., affinity for carbonyl groups. Thus their action may be more specific and they may be ultimately more useful than agents which act in a general way to suppress the whole oral flora.

The significance of in vitro bacteriological studies on known strains of caries-producing streptococci is two-fold. First, any biochemical differences between these organisms and the non-cariogenic streptococci may be exploited by the use of very specific inhibitors which block a particular enzymatic step only. Secondly, a knowledge of differences between these two kinds of streptococci should lead to a better understanding of the actual steps involved in the caries process in humans.

Proposed Course of Project:

Further tests of metabisulfite and other specific carbonyl-binding compounds are under way. Also the addition complexes between bisulfite and certain carbonyl-containing compounds are being tested for their effectiveness as caries-inhibiting agents. Examples of these complexes are formaldehyde sodium bisulfite and acetone sodium bisulfite. Comparative studies on the bacteriology and vitamin content of the rats' cecum when fed bisulfite-containing diets are also in progress.

Comparative nutritional and metabolic studies on the cariogenic and noncariogenic streptococci from the rat and hamster are being extended. Present studies are focused on carbohydrate and fatty acid metabolism. Further studies will include the amino acids and vitamins. Also an attempt will be made to apply findings on animal strains to a study of human streptococci.

Part B included Yes

Serial No. NIDR-6

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Jordan, H. V., Fitzgerald, R. J., and Bowler, A. E. Inhibition of Experimental Caries by Sodium Metabisulfite and its Effect on the Growth and Metabolism of Selected Bacteria. J. Dental Res., 39: 116-123, 1960.

Fitzgerald, R. J., Jordan, H. V., Scott, D. B., and McCann, H.G. Dental Calculus and Antibiotics in the Rat. Arch. Oral. Biol., 2: 85-86, 1960.

Fitzgerald, R. J., Jordan, H. V., and Stanley, H. R. Experimental Caries and Gingival Pathologic Changes in the Gnotobiotic Rat. J. Dental Res., 39: 923-935, 1960.

Jordan, H. V., Fitzgerald, R. J., and Berger, N. D. Carbonyl-binding Compounds as Inhibitors of Experimental Caries in Rats. J. Dental Res., in press.

Honors and Awards relating to his project:

None

Serial No. NIDR-7
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Biological, Chemical, and Physical Properties of Endotoxins from Oral Bacteria

Principal Investigators: Dr. S. E. Mergenhagen
Dr. A. A. Rizzo

Other Investigators: Dr. E. G. Hampp

Cooperating Units: American Dental Association

Man Years (calendar year 1960):

Total:	2 3/4
Professional:	1
Other:	1 3/4

Project Description:

Objectives:

1. To determine which members of the oral microbiota possess endotoxins which could be operative in the pathogenesis of periodontal disease.

2. To analyze the mechanism of action of these endotoxins on host tissue and to study the role they may play in the pathogenesis of experimental infections with oral bacteria.

3. To analyze, by physical and chemical means, the nature of these endotoxins.

Methods Employed:

Tryptic digestion and the two-phase phenol-water extraction of Westphal have been applied to oral bacteria with a view of releasing soluble endotoxins from the cells. Toxicity of isolated bacterial fractions was tested by intracutaneous and intravenous injection in rabbits and intraperitoneal injection in mice. Pyrogenic activities of endotoxic preparations were tested in rabbits. Development of lesions from primary injections in rabbits was followed grossly and histologically. Similar observations were made on rabbits given a second injection intra-

Part A (continued)

venously to elicit the localized Shwartzman reaction. Toxicity for mice was calculated as the 50 per cent lethal dose. In addition, the effects of endotoxin on oral tissues was investigated by injecting these preparations intramucosally in the gingival-palatal area of the rabbit. Both direct toxicity to oral mucosa and Shwartzman reactions in oral mucosa were followed histologically. The effects of intramucosal injection were further studied by measuring systemic temperatures and examining regional lymph nodes draining the injected oral sites.

Chemical and physical analysis of these lipopolysaccharide endotoxins utilizes paper chromatography and specific colorimetric tests for polysaccharide elements, ultracentrifugation to determine the molecular properties of the material, and chemical fractionation of the endotoxin to determine whether the toxic moiety is really only a part of the whole complex isolated by the phenol-water method.

Major Findings:

1. Tryptic digestion of strains of Veillonella, Selenomonas, and Fusobacterium released soluble endotoxins, as demonstrated by their production of inflammatory lesions with ischemic necrosis on intracutaneous injection in rabbits and by their ability to produce the dermal Shwartzman reaction. That these reactions could not be elicited by all bacteria indigenous to the oral cavity was shown by the negative results with tryptic digests of oral anaerobic streptococci and diphtheroid bacilli.

2. Endotoxins, isolated by the phenol-water extraction procedure, have been extracted from oral strains of Veillonella, Selenomonas sputigena, Bacteroides melaninogenicus, Fusobacterium nucleatum, small oral treponemes, Borrelia vincentii and Borrelia buccalis, but not from viridans streptococci. These endotoxins produced intracutaneous inflammatory necrotic lesions, prepared skin sites for the local Shwartzman reaction, were pyrogenic in rabbits, and were lethal for mice. In addition, the endotoxin from Veillonella strains (others not tested) produced inflammatory necrotic lesions in the oral mucosa of the rabbit and prepared this tissue for the local Shwartzman reaction. These effects were obtained with as little as 1 microgram of endotoxin. A comparison of the response of oral mucosa and of skin to similar local injections of endotoxin revealed that the gross and histologic manifestations following this specific type of insult were basically similar in both tissues. It was observed, however, that an intramucosal injection of 1 microgram produced temperature elevation, whereas 25 times this dose given intracutaneously was not pyrogenic.

Part A (continued)

3. The lipopolysaccharide-containing endotoxin fraction, isolated by the phenol-water procedure from veillonella cells, has been characterized chemically as follows: it contained 10 per cent Kjeldahl nitrogen, 4 per cent protein, 4.2 per cent phosphorus, 6 per cent chloroform-soluble lipid after acid hydrolysis, 48 per cent nucleic acid.

Nucleic acid contamination of the above endotoxins could be eliminated by preliminary treatment of the bacterial cells with 0.5 per cent formol. This procedure proved useful for getting highly toxic preparations free of nucleic acid. Veillonella endotoxin so-prepared had low contents of nitrogen (0.4 per cent), phosphorus (0.5 per cent) and protein (1.8 per cent). Concomitant with the reduction in these chemical components, the minimum lethal dose (LD_{50}) for mice and the minimum effective preparatory dose for the local Shwartzman reaction were reduced. Analytical ultracentrifuge data indicate that this endotoxin is polydisperse and has several components of varying molecular weight. However, products separated in a Sephadex column indicate that the fractions with the greatest molecular weight (>50,000) are responsible for the toxicity of the whole fraction tested. Saccharide components thus far identified in veillonella endotoxin have been glucosamine, galactosamine, and a sialic-acid like component. The hexosamine components were identified colorimetrically and by ion-exchange chromatography. Sialic acid was identified by the thiobarbituric acid assay method and also by paper chromatography with known references of N-acetylneuraminic acid and N-glycolylneuraminic acid. Sialic acid has been found also in the endotoxin of Selenomonas sputigena but not in the others.

Significance to Dental Research:

The demonstration by chemical and biological means of endotoxins in certain strains of oral bacteria, especially those which are found in greater abundance in the oral cavity in pathologic processes of oral soft tissues, supports the frequently advanced speculation implicating toxic components of the oral microbiota in the inflammatory, necrotic phases of periodontal disease. Evidence for the marked susceptibility of gingival tissue to these toxic components was demonstrated.

Proposed Course of Project:

To continue our investigations as outlined above with particular reference to mechanisms of action of endotoxin on host tissue, availability of oral tissues to such bacterial products, and a chemical, physical, and immunologic study of such endotoxins.

Part B included Yes

Serial No. NIDR-7

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Mergenhagen, S. E. Endotoxic Properties of Oral Bacteria as Revealed by the Local Shwartzman Reaction. J. Dental Res. 39: 267-272, 1960.

Rizzo, A. A. and Mergenhagen, S. E. Local Shwartzman Reaction in Rabbit Oral Mucosa with Endotoxin from Oral Bacteria. Proc. Soc. Exp. Biol. and Med. 104: 579-582, 1960.

Honors and Awards relating to this project:

None

Serial No. NIDR-8
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Bacteremias Following Exodontia and Periodontal Treatment

Principal Investigator: Dr. T. A. Nevin

Other Investigators: Mr. M. Rogosa
Dr. E. G. Hampp
Dr. R. J. Fitzgerald
Dr. A. Howell, Jr.
Dr. H. V. Jordan

Cooperating Units: American Dental Association

Man Years (calendar year 1960):
Total: 3/4
Professional: 1/4
Other: 1/2

Project Description:

Objectives:

To relate those organisms isolated from the peripheral blood during dental surgery to those isolated from pathological materials obtained from the site of surgery in the same patient. This is a continuation of NIDR-18, 1959.

Methods Employed:

1. Standard bacteriological methods, including 6 tolerance tests, 11 chemical tests, and 26 fermentation reactions, have been used to identify the organisms isolated from oral samples.

2. Upon completion, these data were compared with those obtained similarly from the study of blood isolates in order to show any relationships.

Major Findings:

1. Approximately 180 strains, isolated from blood, have been characterized.

Part A (continued)

2. Approximately 250 strains, isolated from oral samples, have been processed. Among these are about 100 strains of streptococci and diphtheroids isolated in Dr. Fitzgerald's laboratory, and 30 strains of micrococci isolated in Dr. Jordan's laboratory. An additional 30 strains of filamentous organisms have been isolated and characterized in Dr. Howell's laboratory.

3. Two hundred fifty-five strains of organisms, isolated from oral pathological materials, and from the blood of patients undergoing oral surgery were selected for comparison. The preponderance of strains were obtained from oral sources. These were chosen in order to increase the probability of recovering the same, or very similar organisms, from both sources in the same patient. When comparable strains were obtained from each source in the same patient, they appeared closely similar or identical within the probable limits of error of each test used.

In periodontally afflicted patients, organisms similar to those recovered from oral lesions during presurgical treatment, were recovered from the blood during the actual gingivectomies, which were performed as much as ninety days later. When extractions were performed, it was more usual to recover the same, or similar, organisms from the oral lesion and the blood, at the same time.

Significance to Dental Research:

1. The data presented in this, and in previous reports, indicate that a wide variety of organisms are recoverable from the blood of patients undergoing oral surgery provided that adequate cultural methods including several culture media are used.

2. The persistence of organisms in or upon afflicted gingival tissues for extended periods of time following presurgical treatment suggests a possible explanation for the chronicity of periodontal disease.

3. These data should be valuable in designing future studies of the immunological and physiological aspects of infection in dental and related diseases.

Proposed Course of Project:

This project is completed.

Part B included Yes

Serial No. NIDR-8

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Rogosa, M., Hampp, E. G., Nevin, T. A., Wagner, H. J., Driscoll, E. J., and Baer, P. N. Blood Sampling and Cultural Studies in the Detection of Postoperative Bacteremias. J. Am. Dental Assoc., 60: 171-180, 1960.

Nevin, T. A., Rogosa, M., and Hampp, E. G. A Comparison of Blood and Oral Isolates Obtained During a Study of Postoperative Bacteremias. J. Dental Res., in press.

Honors and Awards relating to this project:

None

Serial No. NIDR-9
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on the Physiology of Oral Fusobacteria and on the Proteolytic Enzymes of Microbial Origin

Principal Investigator: Dr. R. R. Omata

Other Investigators: Dr. E. G. Hampp

Cooperating Units: American Dental Association

Man Years (calendar year 1960):
Total: 1
Professional: 3/4
Other: 1/4

Project Description:

Objectives:

The investigation of the physiology of several members of the oral microflora and the nature of their products, specifically, proteolytic enzymes as factors in periodontal diseases.

Methods Employed:

1. Accepted methods and procedures for the isolation and classification of microorganisms were employed. Biochemical activities were correlated with cellular and colonial morphology to differentiate types of oral fusobacteria.

2. Proteolytic activity of the oral spirochetes was tested on various types of substrates. The proteolysis in various cultures was measured by the decrease in total protein and the increase of α-amino acids, and correlated with direct cell counts at various stages of growth.

3. Extracellular proteolytic enzymes, specifically collagenase and proteinase, elaborated by active cultures of Clostridium histolyticum, were fractionated by salting out with ammonium sulfate and by chromatographic methods employing carboxymethyl-cellulose adsorbent columns. These fractions were assayed with newly developed methods.

Serial No. NIDR-9

Part A (continued)

Major Findings:

1. Among the 25 strains of oral fusobacteria studied, 3 main types or species were found, Fusobacterium nucleatum and F. polymorphum were biochemically and culturally similar; however, they differed in their cellular morphology. The third type, F. fusiforme, is very different from the others in morphology and physiology. The latter type or species closely corresponds or is identical to an earlier described oral bacterium, Leptotrichia buccalis, Trevisan.

2. The proteolytic activity of certain oral spirochetes was investigated during growth in Huntoon's broth with and without ascitic fluid. Results showed that the small oral treponeme is enzymatically active during its early growth phase and also exhibits peptidase activity. Borrelia vincentii showed less proteolytic activity during its growth phase.

3. With the use of carboxymethylcellulose chromatographic columns employing a pH gradient elution from pH 4.6 to 8.5, it has been possible to obtain 3 peaks of active fractions from the crude proteolytic enzymes prepared from active cultures of Clostridium histolyticum, and to purify the collagenase activity 5 to 10 fold with this new method.

Significance to Dental Research:

The present investigation is based on the concept of the role of different groups of microorganisms and their metabolic products as contributory factors in the initiation or aggravation of primary periodontal disease. There are implications that various bacteria and their enzymes may have an integral relationship in the etiology of oral diseases, and a basic understanding of these factors should be a fruitful approach.

Proposed Course of Project:

Project discontinued because of transfer of principal investigator.

Part B included Yes

Serial No. NIDR-9

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Omata, R. R., and Braunberg, R. C. Oral Fusobacteria, J. Bacteriol., 80: 737-740, 1960.

Omata, R. R., and Hampp, E. G. Proteolytic Activities of some Oral Spirochetes, J. Dental Res., in press.

Honors and Awards relating to this project:

None

Serial No. NIDR-10
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Immunological, Biochemical, and Pathological Factors in the Taxonomy and Ecology of Oral Bacteria.

Principal Investigator: Mr. M. Rogosa

Other Investigators: Dr. M. E. Sharpe
Dr. G. J. Franklin
Dr. J. E. Ford
Dr. G. C. Cheeseman

Cooperating Unit: National Institute for Research in Dairying
University of Reading, England

Man Years (calendar year 1960):
Total: 1
Professional: 1
Other: 0

Project Description:

Objectives:

To determine the immunological, biochemical, and pathological characteristics of the veillonellae, lactobacilli, diphtheroids, and other oral microorganisms. This project is a continuation of NIDR-22 (1959).

Methods Employed:

1. Antisera, prepared in rabbits against bacterial cells, are tested against the organisms or antigens extracted from them by the techniques of agglutination, precipitation, and selective antibody absorption.

2. Biochemical characteristics are determined by standard and newly developed procedures.

3. Pathogenicity studies are conducted in appropriate animal hosts, inoculated by various routes.

Serial No. NIDR-10

Part A (continued)

Major Findings:

1. Serology: In addition to the new group antigen for Lactobacillus salivarius, a second new group antigen appears to be present in a probable new species of Lactobacillus from the hamster. A unique antigen, which does not react with antisera prepared against Veillonella from man, the rat and rabbit, was found in all Veillonella from the hamster.

2. Biochemical Studies: It was found that nitrate reduction, particularly by the lactobacilli but also by other organisms, was rigorously dependent on the maintenance of a relatively high pH, i.e., neutral or slightly alkaline conditions. Conditions for controlling pH in nitrate reduction and testing for end products were worked out. Improved media and conditions was found for testing nutritional requirements of the lactobacilli. L. bulgaricus was prevented from growing by small amounts of folic acid or folinic acid and this organism, therefore, should prove useful in further studies of the metabolically "active" form of folic acid in physiological studies.

An aberrant strain of L. acidophilus was found to require an unknown growth factor present in distillers' solubles, which also promote the growth of the chick in some experiments. The growth factor for L. acidophilus was proved to be any one of various deoxyribosides. This work was done in collaboration with Dr. J. E. Ford at the National Institute for Research in Dairying, England.

Chromatographic studies of whole cells of diphtheroids in collaboration with Dr. G. C. Cheeseman at the National Institute for Research in Dairying, were repeated with results again indicating only quantitative differences in the total amino acid pool of the cells.

3. A symposium paper on the taxonomy of the lactobacilli (Journal of Applied Bacteriology, 22: 329-340, 1959) has aroused the interest of the Bergey's Manual Editorial Committee, who have indicated that the next edition of Bergey's Manual of Determinative Bacteriology will follow the authors' main outlines for the genus Lactobacillus. Also, an international subcommittee of the International Committee on Bacterial Nomenclature is being formed with the principal investigator as a member.

Serial No. NIDR-10

Part A (continued)

Significance to Dental Research:

Since systematic immunological and pathogenicity studies are lacking, these studies are important in differentiating organisms; in increasing knowledge of the structure of the cell; and in determining the pathogenic potential of the oral microbiota.

Proposed Course of Project:

The immediate future will be devoted to studies of the immunology and chemical composition of the diphtheroids, better methods for their selective isolation, and studies of the PPLO or L forms of bacteria as a stage in the life cycle of bacteria. Further antigenic and chemical analyses of cell components of the genus Veillonella in relation to their pathogenic potential are indicated.

Part B included Yes

Serial No. NIDR-10

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Rogosa, M. and Sharpe, M. E. An Approach to the Classification of the Lactobacilli. J. Appl. Bacteriol., 22: 329-340, 1959.

Rogosa, M. and Sharpe, M. E. Species Differentiation of Human Vaginal Lactobacilli. J. Gen. Microbiol., 23: 197-201, 1960.

Rogosa, M. Experimental Conditions for Nitrate Reduction by Certain Strains of the Genus Lactobacillus. J. Gen. Microbiol., in press.

Rogosa, M., Hampp, E.G., Nevin, T.A., Wagner, H.M., Driscoll, E. J., and Baer, P. N. Blood Sampling and Cultural Studies in the Detection of Postoperative Bacteremias. J. Am. Dent. Assoc., 60: 171-180, 1960.

Nevin, T. A., Rogosa, M., and Hampp, E. G. A Comparison of Blood and Oral Isolates Obtained During a Study of Postoperative Bacteremias. J. Dental Res., in press.

Man de, J. C., Rogosa, M., and Sharpe, M.E. A Medium for Cultivation of Lactobacilli. J. Appl. Bacteriol., 23: 130-135, 1960.

Honors and Awards relating to this project:

Two years as resident visiting scholar, University of Reading, England; returned to U.S.A. on August 9, 1960.

Serial No. NIDR-11
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Manifest and Inapparent Viral Infections of Oral Tissues.

Principal Investigator: Dr. H. W. Scherp

Other Investigators: Dr. R. J. Fitzgerald

Cooperating Units: None

Man Years (calendar year 1960):
Total: 3
Professional: 1/2
Other: 2 1/2

Project Description:

Objectives:

1. To test for the presence of inapparent viruses in the tissues of "germfree" and conventional animals.

2. To isolate, identify, and determine the etiological significance of viruses from various lesions of the oral mucosa of undetermined etiology.

This project is a continuation of NIDR-23 (1959).

Methods Employed:

Samples containing tissue cells are frozen and thawed to liberate intracellular viruses. To test for viruses, the samples are inoculated into standard tissue cultures of rabbit kidney cells, monkey cells, HeLa cells, mouse L cells, chicken embryo cells, and human skin cells. After incubation, cultures are frozen, thawed and transferred to a fresh tissue culture; serial transfers are made. In addition, specimens of biopsy tissue are maintained in tissue culture medium for protracted periods to allow development of latent viruses. Evidence for a virus is sought by microscopic examination for cytopathologic changes in the cultured cells and by hemagglutination and hemadsorption tests of the culture fluids with guinea pig and chicken erythrocytes.

Part A (continued)

Isolates of viruses are identified by neutralization tests with specific antisera, when applicable. Refined antigenic analysis of related viruses is accomplished by comparison of the kinetics of their neutralization by homologous and heterologous antisera using a plaque-count method for quantitation of virus. Results are expressed as a pseudomonomolecular rate constant, K; homology is evidenced by the highest K values.

Major Findings:

1. Serologic differentiation of strains of herpes simplex virus. Preliminary results reported in NIDR-23, 1959, showed the feasibility of distinguishing between strains of herpes virus by determination of their K values with homologous and heterologous antisera. Accordingly during 1960 effort has been concentrated on working toward an antigenic schema based on the necessary reciprocal neutralization tests with 15 strains of herpes virus (4 stock strains and 11 strains freshly isolated from patients; each test repeated at least once) and rabbit antisera to 5 of them. In order to test the applicability of the provisional schema that has eventuated, we have recently incorporated into the experimental plan antisera against 6 more of these strains and have isolated 6 additional strains of herpes virus, including one from a primary herpetic infection, several from recurrent attacks in the same individuals, and the classical HF strain, which has served as the prototype in many past studies since the 1920's.

The 15 strains studied in detail fell quite distinctly into 4 antigenic groups (A-D): 5 in group A, 2 in group B, 3 in group C, and 5 in group D. The homologous and heterologous K values of group D strains, however, indicate antigenic overlapping with all of the other strains. This observation is of course consistent with the well-known antigenic homogeneity of herpes virus as determined by conventional methods. Of particular interest was the finding that the HF strain, though neutralized rapidly by homologous antiserum, was neutralized so slowly by the heterologous antisera as to indicate almost complete immunologic dissimilarity. Evidently this strain has undergone antigenic mutation during the course of its innumerable passages over the years through rabbits, mice, and chick embryos.

2. A cytopathic agent from the salivary glands of rats. Previously we reported (NIDR-23, 1959) some of the properties of a possibly viral agent found in the salivary glands, but not in any of a variety of other organs and tissues, of both conventional and germfree rats. Pending accumulation of an adequate new

Serial No. NIDR-11

Part A (continued)

supply of salivary glands from germfree rats, this project was de-emphasized during 1960 in favor of the serologic study of herpes virus. The agent has now been recovered from a total of 29 of the 39 germfree salivary glands tested. Its heat inactivation (56°C, 60 minutes) has been demonstrated.

Significance to Dental Research:

Oral soft tissue lesions of unestablished but possibly viral etiology are frequently encountered by the dentist. The still incompletely understood phenomena of latency and activation of herpes virus are accordingly of direct dental interest; they also provide prototypes of fundamental viral relationships. The availability of a method to distinguish between strains of herpes virus will make possible elucidation of previously inaccessible phases of the ecology of this ubiquitous agent. Study of inapparent viral infection in otherwise germfree animals affords another approach to the study of the consequences of such infections on "normal" physiology.

Proposed Course of Project:

The previously described studies of serological differentiation of strains of herpes virus and of cytopathogenic agent from rat salivary glands will probably be brought to consummation during 1961.

Part B included Yes

Serial No. NIDR-11

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Ship, I. I., Ashe, W. K., and Scherp, H. W. Recurrent "Fever Blister" and "Canker Sore": Tests for Herpes Simplex and other Viruses with Mammalian Cell Cultures. Arch. Oral Biol., in press.

Honors and Awards relating to this project:

None

Serial No. NIDR-12
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Nutrition and Metabolism of Lactic Acid Bacteria

Principal Investigator: Dr. T. Shiota

Other Investigators: Dr. J. E. Folk
Dr. J. Mauron

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1 1/4
Professional: 1/2
Other: 3/4

Project Description:

Objectives:

To study amino acid, peptide and vitamin metabolism by lactic acid bacteria with specific attention to enzymatic synthesis of folic acid-like compounds by purified extracts of Lactobacillus plantarum and amino acid and peptide utilization by L. plantarum and Leuconostoc mesenteroides.

Methods Employed:

1. The determination of folic acid-like compounds was carried out by microbiological assay, chromatography and spectrophotometry.

2. The utilization of amino acids and peptides was ascertained by inhibition analyses of growth response curves.

Major Findings:

1. Folic Acid

Previously, we assumed that tetrahydrofolic acid was the immediate reaction product of the coupling reaction between 2-amino-4-hydroxy-6-hydroxymethyl-tetrahydropteridine (THP) and p-aminobenzoylglutamate in the enzymatic synthesis of folic acid-

Part A (continued)

like compounds by extracts of Lactobacillus plantarum. Since then, however, we found evidence that the immediate product is dihydrofolic acid, which is subsequently reduced by reduced diphosphopyridine nucleotide or triphosphopyridine nucleotide to tetrahydrofolate. Consequently, 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine (DHP) was prepared. It was characterized by (a) the stoichiometric consumption of two atoms of hydrogen per mole of pteridine, (b) demonstration by iodometric titration of two atoms of hydrogen per mole of pteridine and (c) the similarity of its ultraviolet absorption spectrum to that of another dihydropteridine, dihydroxanthopterin. DHP was found to be 2-3 times more active than THP on a molar basis in serving as the pteridine in the coupling reaction. Dihydrofolate was identified chromatographically. It is reduced by dihydrofolate reductase to tetrahydrofolate.

2. Amino Acids and Peptides (with Drs. J. E. Folk and J. Mauron).

We have demonstrated that a lysine antagonist, L-S-(β-aminoethyl)cysteine (AEC) was a potent competitive inhibitor of L-lysine utilization by Leuconostoc mesenteroides and Lactobacillus plantarum. The peptides, L-lysylglycine and glycyl-L-lysine were more active than lysine in reversing AEC inhibition with L. mesenteroides. With L. plantarum, AEC inhibition was not observed with L-lysylglycine, glycyl-L-lysylglycine and at certain concentrations of glycyl-L-lysine.

Recently, the inhibitor in a peptide form, L-S-(β-aminoethyl)-cysteinylglycine (AEC-G) was prepared. AEC-G was found to be as potent an inhibitor as AEC for lysine utilization for both organisms. Unlike AEC, AEC-G was a very effective competitive inhibitor of peptide utilization by both organisms. In other words, in the presence of AEC-G, peptides containing lysine behaved similarly to lysine.

Significance to Dental Research:

Studies of amino acid and vitamin metabolism of lactic acid bacteria will add information concerning the ability of these organisms to flourish in the oral cavity. This should help us to understand the role they play in the complex microbial chemical processes related to certain oral diseases.

Proposed Course of Project:

Part A (continued)

1. Folic Acid

We anticipate continuance of this project. The requirement for ATP in the enzymatic synthesis of folic acid-like compounds is as yet not fully understood. We have yet to obtain a sufficiently pure preparation of a phosphorylated derivative of a pteridine which may be the intermediate that explains the ATP requirement. We have observed that cells of L. plantarum can synthesize pteridines. Glucose and serine metabolism have been found to be related to this. Hence we intend to study the pathway of pteridine synthesis.

2. Amino Acids and Peptides

At the present, another peptide containing AEC is being prepared. With AEC and its peptide, we will investigate the incorporation of lysine and its peptide into protein of bacterial cells.

Part B included No

Serial No. NIDR-13
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar year 1960

Part A

Project Title: Studies of Interactions Between Oral Organisms.

Principal Investigator: Dr. T. A. Nevin

Other Investigators: Dr. H. V. Jordan
Dr. P. N. Baer
Dr. N. Littleton

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2 1/2
Professional: 3/4
Other: 1 3/4

Project Description:

Objectives:

1. To study the biochemical bases of the dynamic equilibria which determine the complex nature of the flora of the mouth.

2. To investigate the occurrence of, and the nutritional basis of, symbiosis among the more common microorganisms in the mouth.

3. To employ more fastidious organisms, such as spirilla, various filamentous forms and spirochetes, in order to discover less fastidious microbes whose products are required by, or enhance, the growth of the more exacting types.

Methods Employed:

1. The principal method is a modified auxanographic technique. It employs suitably formulated culture media, seeded with a selected bacterium which serves as an indicator of reaction. Standard amounts of test culture suspensions are pipetted to sterile filter paper disks which are then placed on the surface of the seeded medium. Controls are effected by the use of appropriate reference solutions in place of the test culture. Thus, quantitative as well as qualitative data are obtainable.

Serial No. NIDR-13

Part A (continued)

2. Standard or modified chemical, physical or microbiological methods are used to identify common metabolites. Specifically used for current work were:

A. Quantitative Sakaguchi reaction for arginine.
B. The Ninhydrin Reaction of Chinard, and the Clostridium septicum decarboxylase of Gale for ornithine.
C. Nessler's reaction for free NH_3.
D. The diacetyl reaction of Knivett for citrulline.
E. Manometric methods for CO_2.

Major Findings:

1. The partially defined medium described previously has been used for preliminary studies of the physiology of Borrelia vincentii. Thus far, indole and NH_3 have been identified as metabolic end-products, and indirect evidence indicates that lactic acid may be produced also. With respect to NH_3 production, the guanido group of L-arginine was revealed as the primary source. Glutamine is deaminated also, but at a much lower rate. Since the arginine dihydrolase system of the streptococci is known to be exergonic, the demonstration of such a system in a spirochete might aid in explaining its energy pathways. If an arginine dihydrolase were active, then each mole of arginine should be degraded to 2 moles of NH_3, 1 of CO_2, and 1 of L-ornithine, with no intermediate formation of urea.

Experimentally, 1 micromole of arginine was converted to 1 micromole each of NH_3 and citrulline by resting cell suspensions. When ADP was added in micromolar equivalents, however, the theoretical conversion described above was approximated. The intermediary position of citrulline was determined by substituting this amino acid for arginine, with and without added ADP. In the absence of the coenzyme, no reaction occurred, whereas in its presence, 1 micromole each of NH_3, CO_2 and ornithine were recovered. It is evident, therefore, that a true dihydrolase system is present in B. vincentii. It differs from those of other bacteria in that each step in the over-all reaction can be demonstrated with living cells. Ordinarily, the separate steps must be demonstrated with cell-free enzyme preparations. Further, in B. vincentii, an exogenous source of ADP must be furnished in order for the ureidase reaction to occur.

The fate of the ADP in the living system is not clear. The coenzyme is broken down by the spirochete but is apparently not assimilated; neither is any appreciable amount of inorganic

Part A (continued)

phosphate assimilated, unless both ADP and either arginine or citrulline are present. In the latter instance, 1 less micromole or inorganic phosphate is recovered from the menstruum, for each micromole of amino acid metabolized. Part of the "bound" phosphate was recoverable by barium fractionation of the cells and of the menstruum. The balance was recovered by hydrolyzing the cells in concentrated H_2SO_4.

The steps of the reaction are as follows:

1. Arginine ——————> Citrulline + NH_3

2. Citrulline ——ADP——> ornithine + CO_2 + NH_3

3. ADP + $HPO_4^{=}$ ——————>ATP ——————>other unidentified phosphorylated compounds.

2. Further studies of the ascitic-fluid growth factor have been conducted. The factor has been demonstrated in mouse ascitic fluid, milk whey, and veal heart infusion as well as canine ascitic fluid. In all instances, the factor has been shown to be heat stable (100°C, 10 minutes) acetone insoluble and pH 4.5 soluble.

Qualitative studies of the partially purified factor, including the biuret, ninhydrin, Millon, and lead-acetate tests suggest that it is a peptide-containing tyrosine and a sulfur-containing moiety, probably cystine, as integral parts of the chain.

3. In order to learn more about the interactions between tissues and their indigenous flora, aqueous extracts of several animal tissues, human skin, and normal and diseased human gingival tissue, were prepared and tested qualitatively for their effect on the growth of 5 frequently encountered oral bacteria (Veillonella, Staphylococcus, diphtheroid, Streptococcus, Lactobacillus), under aerobic and anaerobic conditions. An auxanographic method was used. (J. Dent. Res., 37: 427; 37; 527,1958).

Of the organisms tested, only a Veillonella and a Staphylococcus were inhibited. This was evidenced by a faint zone of inhibition around the filter paper disc and no colony development in the subjacent medium, indicating that the inhibiting substance did not diffuse readily. There were no apparent differences between normal and diseased tissue samples. There is an indication also that the type of tissue may have some bearing on the growth of organisms since only extracts of gingivae, skin, and

Part A (continued)

duodenum were inhibitory under aerobic and anaerobic conditions, whereas the Veillonella was unaffected and the Staphylococcus was stimulated under aerobic conditions by extracts of internal organ tissues. The stimulation was manifested as zones of dense growth up to 2 cm in diameter around the discs. The growth of the diphtheroid was affected similarly. Since 16 instances of growth stimulation occurred during aerobic incubation as compared with 4 during anaerobic incubation, it is evident that the extracts contained diffusible reducing substances.

The Streptococcus was stimulated aerobically and anaerobically by extracts of monkey gingivae and duodenum, and aerobically by monkey skin; and the Lactobacillus, anaerobically by rat liver extract, but the growth of neither of these organisms was affected by the other extracts tested.

Significance to Dental Research:

1. Studies such as that herewith reported are natural sequelae to those concerning the nutrition of single organisms. Knowledge of the nutritional requirements of multiple cultures will eventuate in the more complete understanding of biological microcosms.

2. An understanding of the mechanisms whereby the nature of the oral flora is determined in health, will enable the more certain recognition of changes therein due to, or having etiological significance in, oral diseases.

Proposed Course of Project:

This project has been interrupted as of 31 December 1960 by the departure of the principal investigator.

Part B included Yes

Serial No. NIDR-13

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Nevin, T. A., Hampp, E. G., and Duey, B. V. An Interaction Between Borrelia vincentii and an Oral Diphtheroid. J. Bacteriol., 80: 783-786, 1960.

Nevin, T. A., and Jordan, H. V. Natural or Derived Materials which Replace Ascitic Fluid for the Growth of an Oral Spirochete. J. Dental Res., in press.

Honors, and Awards Relating to this Project:

None

NATIONAL INSTITUTE OF DENTAL RESEARCH

Individual Project Reports
Calendar Year 1960

BIOCHEMISTRY:

14. H. Blumenthal: Metabolism of Oral Flora and Salivary Glands.

15. M. S. Lewis: Physical Chemistry of Proteins.

16. A. H. Mehler: Enzyme Structure and Mechanism of Action.

17. K. A. Piez: Biochemistry of Amino Acids and Proteins in Mammalian Cell Cultures.

18. G. R. Martin & S. E. Mergenhagen: Studies on Collagen. I. Studies on Collagenolysis. II. Studies on In Vivo Calcification.

19. E. C. Wolff: Metabolism of Amino Acids, Protein Biosynthesis, and Related Enzymology. I. Blood Clotting: Metabolism of Peptides and Enzymatic Mechanisms. II. Proteolytic Enzymes: Kinetics and Mechanism of Action.

20. J. E. Folk: Protein and Amino Acid Chemistry. I. Proteolytic Enzymes: Chemical and Molecular Studies. II. Blood Clotting: Molecular Mechanism and Enzymology.

21. R. C. Likins: Biochemical and Biophysical Studies of Calcification.

22. I. Zipkin: Biochemistry and Physiology of the Salivary Glands and Their Secretions.

23. I. Zipkin: The Role of Citrate in the Calcifying Tissues.

24. C. T. G. King: Correlation of Clinical Oral Health Findings to Biochemical Stress-related Factors in Personnel Wintering in Antarctica.

25. C. T. G. King: The Effect of Prenatal Factors on Fetal Development and Dental Caries in the Progeny.

26. G. R. Martin: Studies on Ascorbic Acid. I. The Transport of Ascorbic Acid. II. Role of Ascorbic Acid in Formation and Maintenance of Dental Structures.

27. F. J. McClure: The Relation of Dietary Minerals and Protein to Dental Caries.

NATIONAL INSTITUTE OF DENTAL RESEARCH

Individual Project Reports
Calendar Year 1960

BIOCHEMISTRY (Continued):

28. R. H. Larson: Role of Bacteriology and Nutrition in Experimental Dental Caries.

29. K. A. Piez: Analytical and Structural Aspects of Collagen and Proteins of Related Interest in Relation to Function.

Serial No.NIDR-14
1. Biochemistry
2. Enzyme Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Metabolism of Oral Flora and Salivary Glands

Principal Investigators: Subproject A: Dr. H. Blumenthal
Subproject B: Dr. H. Blumenthal and Dr. T. Shiota

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):

Total:	3
Professional:	1½
Other:	1½

Project Description:

Subproject A:

Metabolism of adenosine and some phosphorylated derivatives by the rat's submaxillary glands.

Objectives:

To study deamination and phosphate-release by rat submaxillary preparations incubated in the presence of purine derivatives.

Methods Employed:

Standard methods for (a) the extraction of tissues, (b) ion exchange and paper chromatography, (c) analysis for metabolites, and (d) spectrophotometry.

Major Findings:

Studies of the metabolic breakdown of adenosine triphosphate, adenosine diphosphate, adenylic acid and adenosine by rat submaxillary gland homogenates have been continued. With these homogenates deamination occurred in all these substrates and specific activities for the deaminations were determined. The concomitant release of orthophosphate by these preparations was also studied. The possibility of pyrophosphate release is difficult to assess due

to an active pyrophosphatase also present in these preparations. The role of magnesium ion in these preparations was studied since preliminary data suggest it may be unimportant in the deamination of adenosine triphosphate.

Significance to Dental Research:

Basic data are being obtained relative to the metabolism of salivary glands.

Proposed Course of Project:

Further studies on the purification and characterization of the mechanisms by which purine derivatives are metabolized in salivary glands will be pursued.

Subproject B:

Hexosamine metabolism of microorganisms.

Objectives:

To study the metabolism of hexosamines (particularly galactosamine) by microorganisms.

Methods Employed:

Standard methods and procedures for (a) the study of growth of bacteria, (b) isolation of enzymes from bacteria, (c) analyses for metabolites, (d) ion exchange and paper chromatography and (e) spectrophotometry.

Major Findings:

As reported previously, a large number of bacteria have been screened for their ability to grow in media where galactosamine or glucosamine is the sole carbohydrate source. Of the many microorganisms screened, Lactobacillus casei and Lactobacillus bulgaricus proved most promising. It was found that both wet and dry cell preparations of these organisms could liberate ammonia when incubated with the hexosamines as substrate. It was further found that a calf-trachea-hydrolysate preparation or galactose could substitute for galactosamine and still support growth of galactosamine-metabolizing organisms. Sonicates of these cells, tested against galactosamine, were found to be inactive and a variety of cofactors failed to restore activity in the sonicates. Galactosamine-1-phosphate and galactosamine-6-phosphate were then synthesized and tested with cell-free extracts. Galactosamine-6-phosphate was actively deaminated by these preparations and appears to be the primary substrate for this deamination.

Significance to Dental Research:

Hexosamines are constituents of salivary polysaccharides and as such are present in the oral cavity. The mode of action of oral microorganisms on constituents of the saliva may be significant in the etiology of oral diseases.

Proposed Course of Project:

Work has been started on the purification of the enzyme(s) responsible for the deamination of galactosamine. In addition, techniques for separation and identification of postulated breakdown products are being perfected.

Part B included Yes

Serial No. NIDR-14

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Blumenthal, A., Shiota, T., McCann, M. P., and Disraely, M. N. The Cultivation of D-galactosamine-metabolizing Lactic Acid Bacteria. J. Gen. Microbiol. In press.

Serial No. NIDR-15
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Physical Chemistry of Proteins

Principal Investigator: Dr. M. S. Lewis

Other Investigators: Dr. K. A. Piez

Cooperating Units: None

Man Years (calendar year 1960):

Total: 1
Professional: 1
Other: none

Project Description:

Objectives:

To study the physical and chemical properties of collagen and related proteins, particularly with respect to structure and function.

Methods Employed:

Sedimentation data on collagens prepared from the skin of the calf, rabbit, rat, and dog fish shark, and on the gelatins prepared from these collagens were obtained by ultracentrifugation, measurement of the resultant plates, and appropriate calculations. The temperature, dependence and kinetics of denaturation were studied by viscometry and optical rotation. The intrinsic viscosity and rotary dispersion were also determined. Amino acid analysis was performed by ion exchange chromatography.

Major Findings:

To date the major effort has been placed on a study of dog fish shark collagen. This material has not been previously studied; because of its unusual properties it may offer a means of obtaining information on the basic structure of collagen, which cannot be as readily obtained with mammalian collagens. The original studies have characterized the protein with respect to sedimentation coefficients, viscosity, optical rotation, and amino acid composition. The denaturation of the protein was studied in relation to

these properties.

Significance to Dental Research:

Collagen is a major structural protein in teeth, bone, periodontium, and in connective tissue generally, and as such, probably is significantly involved in the normal function and pathology of these tissues. Basic studies of this protein may thus afford a better understanding of function. These results are also significant in the general field of protein structure.

Proposed Course of Project:

Studies on the physical properties of collagens from various sources (and other proteins as the need arises) will continue in the manner described above. Additional techniques will be used to extend the usefulness of the present data. The immediate problem will be to study gelatins resulting from the denaturation of shark skin collagen, to evaluate the relationship between the structure of the individual chains and the whole molecule. Similar studies will also be made of other collagens, and will include an examination of environmental factors and pathology in relation to the properties of collagen.

Part B included Yes

Serial No. NIDR-15

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Piez, K. A., Weiss, E., and Lewis, M.S. The Separation and Characterization of the α- and β-Components of Calf Skin Collagen. J. Biol. Chem. 235:1987-1991, July 1960.

Serial No. NIDR-16
1. Biochemistry
2. Enzyme Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Enzyme Structure and Mechanism of Action

Principal Investigator: Dr. A. H. Mehler

Other Investigators: None

Cooperating Units: NIAMD

Man Years (calendar year 1960):

Total:	1/2
Professional:	1/2
Other:	none

Project Description:

Objectives:

To determine the primary structure of the enzyme aldolase, to determine the nature of linkages between enzyme and substrate, to determine the factors that influence catalytic activity and ascertain the nature of the catalytic process.

Methods Employed:

Pure crystalline enzyme is subjected to various chemical and physical treatments, and amino acid end groups, free amino acids and peptides are determined and the physical properties of the protein are determined with the ultracentrifuge, electrophoresis, and column chromatography. Kinetic assays of enzymic activity are carried out on nature and altered enzyme preparations with various substrates and inhibitors in the spectrophotometer. The spectrophotometric changes produced by combination of enzyme and substrates are measured.

Major Findings:

End-group analysis is compatible with the apparent 150,000 molecular weight of aldolase being the sum of three identical subunits. The combination of dihydroxyacetone phosphate and aldolase appears to be a true enzyme-substrate complex in which the sub-

strate assumes an enolate structure. A kinetic assay for the combination reaction of aldolase has been developed and kinetic constants for the various substrates have been determined. The effect of altering the enzyme with carboxypeptidase has been explained in kinetic terms. The ability of substrates and inhibitors to protect aldolase from digestion by various proteolytic enzymes has been determined, and the results have been interpreted in terms of probable structures of the enzyme.

Significance to Dental Research:

These studies are part of a program to elucidate the basic nature of enzymes. Since all biological processes are fundamentally enzymatic, it is necessary to understand the nature of enzymes in order to obtain an understanding of normal and pathological biological processes.

Proposed Course of Project:

All of the approaches mentioned above will be continued to define additional structural features and catalytic properties of aldolase. Similar enzymes from other sources will be investigated in order to determine which properties may be correlated.

Part B included No

Serial No. NIDR-17
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Biochemistry of Amino Acids and Proteins in Mammalian Cell Cultures.

Principal Investigator: Dr. K. A. Piez

Other Investigators: None

Cooperating Units: Dr. H. Eagle, NIAID

Man Years (calendar year 1960):

Total:	1½
Professional:	½
Other:	1

Project Description:

Objectives:

It is the broad purpose of this project to obtain basic information relative to protein and amino acid metabolism in mammalian cells in culture. Current studies relate to the utilization of protein and the synthesis of cystine.

Methods Employed: Usual laboratory procedures

Major Findings:

The requirement of serum protein in media for cell culture makes the interpretation of certain studies difficult because the protein is not well defined. For this reason its metabolic fate was investigated. A maximum of 3 to 6% of the cell protein of newly formed cells was derived from the protein in the medium. However, dialyzed serum-protein also underwent proteolysis by its own enzymes to yield free amino acids. This may become an important factor in the interpretation of certain experiments particularly when a C^{14} labeled compound is employed, owing to dilution of added amino acid by that released from the protein.

It was found that in cell culture, cystine is made from methionine sulfur and glucose carbon, by the classic pathway. However, even though cystine is synthesized it is still necessary to include it in the medium. It was determined that this was the result of the inability of the cell to maintain its internal pool against

the demands of the medium. In very large cell populations, where this demand can be satisfied by more cells, cystine was no longer required.

Significance to Dental Research:

Cell culture is a relatively new tool suitable for the study of many metabolic processes. These basic studies are important to all branches of the medical sciences. Problems more immediately related to dental research, such as collagen biosynthesis, are amenable to study with these techniques.

Proposed Course of Project:

It is planned to continue these studies along the lines already started. Investigations are underway on some aspects of protein synthesis.

Part B included Yes

PHS-NIH Serial No. NIDR-17

Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Eagle, H., and Piez, K. A. The Utilization of Proteins by Cultured Human Cells. J. Biol. Chem. 235:1095-1097, April 1960.

Eagle, H., Oyama, V. I., and Piez, K. A. The Reversible Binding of Half-Cystine Residues to Serum Protein, and its Bearing on the Cystine Requirement of Cultured Mammalian Cells. J. Biol. Chem. 235: 1719-1726, June 1960.

Piez, K. A., Eagle, H., and Levintow, L. Proteolysis in Stored Serum and its Possible Significance in Cell Culture. Nature 188:59-60, October 1960.

Serial No. NIDR-18
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies on Collagen. I. Studies on Collagenolysis. II. Studies on *in vivo* Calcification.

Principal Investigators: Dr. G. R. Martin (ADA Research Associate) and Dr. S. E. Mergenhagen.

Other Investigators: Dr. A. A. Rizzo and Dr. D. B. Scott

Cooperating Units: None

Man Years (Calendar year 1960):

Total:	2
Professional:	1¼
Other:	3/4

Project Description:

Objectives:

I. To elucidate the events leading to, and causing the breakdown and removal of tissue collagen in normal and pathological conditions.

II. To determine the role of the organic matrix in calcification.

Methods Employed:

I. Collagen from various sources is purified by successive precipitations and extractions. Radioactive collagen is prepared in this same manner from animals which have received C^{14} labeled amino acids. Degradation of the collagen is assayed by viscosimetric methods, as well as by measuring the susceptibility of the collagen to enzymatic attack.

II. Various collagenous and other substrates are implanted into the peritoneal cavity of rats for different periods. Following their removal, these matrices are examined by chemical and physical methods (X-ray, electron microscopic, etc.).

Major Findings:

(a) The urinary excretion of hydroxyproline was elevated in growing animals, in previously scorbutic guinea pigs given ascorbic acid, and in lathyritic animals. In these conditions the amount of soluble collagen is believed to be increased. *In vitro*, enzymatic and viscosimetric studies of the thermal

denaturation of reconstituted collagen indicate that collagen is denatured at temperatures above 30° C if kept in solution. This denatured collagen is readily attacked by a variety of enzymes. These findings suggest that one pathway of collagen breakdown involves the non-enzymatic, thermal denaturation of collagen followed by enzymatic destruction of the denatured collagen.

In addition, using radioactive reconstituted collagens isolated from rat and from rabbit skin, it was demonstrated that the two collagens differ in structure. Specifically, trypsin releases a small amount of material from rat skin collagen but not from rabbit skin collagen. The possibility exists that the collagen molecule isolated from rat skin contains small peptide appendages.

(b) Studies on collagen resorption indicate that rat tail tendon and reconstituted rabbit skin collagen treated with phosphate and also decalcified rabbit dentin mineralized when implanted in the peritoneal cavity of rats. The mineral was found to be hydroxyapatite and the crystals were oriented parallel to the axis of the collagen fiber.

Human oral bacteria which were placed in dialysis tubing and implanted in the peritoneal cavity rapidly developed a mineral deposit which was morphologically indistinguishable from calculus. The method of implanting various substances into the peritoneal cavity was found to provide a convenient system for studies on calcification. Further studies on the mechanisms involved in these processes are in progress.

Significance to Dental Research:

Since collagen is an important constituent of the hard and soft tissues of the oral cavity, a classification of the events involved in its calcification, formation and destruction may contribute to our understanding of pathological conditions in which collagen metabolism may be altered.

Proposed Course of Project:

I. It is planned to examine the ability of various mammalian tissues and enzyme systems to metabolize collagen.

II. Further studies on the biochemical events in calcification are in progress.

Part B included Yes

Serial No. NIDR-18

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Mergenhagen, S. E., Martin, G. R., Rizzo, A. A., Wright, D. N., and Scott, D. B. Calcification of Implanted Collagen. Biochem. Biophys. Acta 43:563-565, October 1960.

Martin, G. R., Mergenhagen, S. E., and Scott, D. B. Relations of Ionizing Groups to the Structure of the Collagen Polymer. Biochem. Biophys. Acta. In Press.

Serial No.NIDR-19
1. Biochemistry
2. Enzyme Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Metabolism of Amino Acids, Protein Biosynthesis, and Related Enzymology. I. Blood Clotting: Metabolism of Peptides and Enzymatic Mechanisms. II. Proteolytic Enzymes: Kinetics and Mechanism of Action

Principal Investigator: Dr. E. C. Wolff

Other Investigators: Dr. J. E. Folk, Dr. K. A. Piez

Cooperating Units: Section on Physical Biochemistry, NIAMD

Man Years (calendar year 1960):

Total:	1 1/4
Professional:	1
Other:	1/4

Project Description:

Objectives:

I. Isolation and characterization of the peptides released from rabbit fibrinogen during clotting; the metabolic origin of the tyrosine-O-sulfate present in one of the peptides.

The biosynthesis and intermediary metabolism of fibrinogen, and factors affecting the enzymatic action of thrombin on fibrinogen.

II. A study of the kinetics of carboxypeptidase B; effects of inhibitors and activators, salt concentration, pH, and temperature on kinetic parameters; substrate specificity and effects of enzyme modifications.

Methods Employed:

I. (a) The isolation of rabbit "co-fibrin" peptides involved a new technique of gel filtration, separation by paper electrophoresis or ion-exchange chromatography, after conventional steps of protein precipitation. Established methods for end-group analysis and automatic amino acid analysis determined the composition of the peptides. For demonstration of tyrosine sulfate a combination of alkaline hydrolysis, ion-exchange resin selection and a special two-

dimensional paper chromatographic system was devised, coupled with ultraviolet spectroscopy after selective acid hydrolysis.

Conventional tracer techniques, gas-flow counting and radioautography, a modified method of charcoal absorption and ion-exchange resin chromatography permitted isolation of aromatic amino acids and determination of specific activities.

II. Modification of the spectrophotometric assay for carboxypeptidase B by using absorption cells of long path length and conditions suitable for kinetic analysis.

Major Findings:

I. The clotting of rabbit fibrinogen by purified bovine thrombin is accompanied by the release of two low-molecular weight peptides, A and B, analogous to the finding in bovine and human systems. These peptides were isolated, characterized, and the amino acid composition, N-terminal and C-terminal residues and minimum molecular weight of each determined. A significant finding was the presence of tyrosine-O-sulfate in one of the peptides and in the residual fibrin as has been reported also in bovine peptide B and fibrin. Radioactive tracer experiments have shown that tyrosine and phenylalanine, probably via tyrosine, are incorporated *in vivo* into the tyrosine sulfate of peptide B of rabbits to about the same extent as into the tyrosine of the remainder of the fibrinogen molecule. Inorganic sulfate appears to be a good precursor for the sulfate ester moiety. In confirmation of the work of others, preliminary experiments with known phenol sulfate ester-forming enzyme systems from rabbit liver have not yet shown any reaction with free tyrosine.

II. Preliminary studies have shown the feasibility of kinetic analysis of the peptidase activity of carboxypeptidase B by the modified spectrophotometric assay. Certain differences in the effects of substrate concentration, inhibition, pH, and salt concentration on this enzymatic activity compared to those on carboxypeptidase A have been noted.

Significance to Dental Research:

I. More complete knowledge of the blood clotting process and of the intermediary metabolism of the proteins and peptides involved in it, should afford opportunity for correction of abnormalities affecting clotting and for a better understanding of the general processes of protein biosynthesis essential to all normal functions.

II. Investigation of the action of a specific proteolytic enzyme, and comparison with a closely related enzyme, contributes to the

understanding of enzymatic processes in general.

Proposed Course of Project:

I. The characterization of rabbit peptides may be extended to sequential analysis, especially in regard to the region around the tyrosine sulfate. Further studies will be directed at elucidating the immediate precursor of the tyrosine sulfate, or the substrate for the sulfate ester formation and the enzymatic processes involved. Development of an experimental system in which the biosynthesis of fibrinogen can be studied more directly in perfused liver preparations, in liver slices or in a completely cell free system, is projected.

The possibility of reversal of the proteolytic action of thrombin, as well as the effects of modifying the structure of fibrinogen or the peptides derived from it either biosynthetically or chemically, on the action of thrombin will be explored. Isolation of a possible intermediate in the clotting process in which only one peptide has been removed from fibrinogen is contemplated.

II. Kinetic studies of the action of carboxypeptidase B, as outlined under Objectives are being carried forward, with comparison of several synthetic peptides as substrates.

Part B included Yes

Serial No. NIDR-15

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Wolff, E. C. and Arnstein, H. R. V. The Metabolism of 6-Aminopenicillanic Acid and Related Compounds by Penicillium chrysogenum and its Possible Significance for Penicillin Biosynthesis. Biochem. J. 76:375-381, Aug. 1960.

Serial No. NIDR-20
1. Biochemistry
2. Enzyme Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Protein and Amino Acid Chemistry. I. Proteolytic Enzymes: Chemical and Molecular Studies. II. Blood Clotting: Molecular Mechanism and Enzymology

Principal Investigator: Dr. J. E. Folk

Other Investigators: Dr. E. C. Wolff
Dr. K. A. Piez

Cooperating Units: Section of Physical Biology, NIAMD

Man Years (calendar year 1960):

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

I. (a) The specificity and mode of action of the carboxypeptidases (b) Purification, molecular and chemical characteristics of carboxypeptidase B and its zymogen.

II. Specificity and mechanism of thrombin-induced conversion of fibrinogen to fibrin; structure and properties of the peptides formed during this conversion.

III. Separation and chemical studies of the genetic species of β-lactoglobulin.

Methods Employed:

I. (a) Studies of the changes in specificity and rate toward synthetic substrates following replacement of native zinc by other first transitional period metal ions have been conducted and are being continued. Other peptide substrates for native carboxypeptidase B, designed so that their rates of hydrolysis may be followed spectrophotometrically, have been prepared.

(b) Classical chemical and enzymatic methods of end-group analysis

have been applied to carboxypeptidase B. Certain steps in the purification of the carboxypeptidase B zymogen have been followed by the spectrophotometric assay procedure.

II. Synthetic peptides containing arginine have been prepared and tested as thrombin substrates. The so-called "active centers" of bovine and human thrombin have been labelled with radioactive phosphate and studies of the sequence of amino acids in these regions are under way.

Chemical and enzymatic methods of end-group analysis have been applied to peptic digests of Peptide B in order to obtain the entire primary structure of this peptide.

III. Attempts are being made to locate the positions and relationships of different amino acid residues in two genetic species of β-lactalbumin, A(I) and B(II), (see project report of Dr. Piez). Preliminary studies involve digestion of lactalbumins and separation of peptides.

Major Findings:

I. (a) Replacement of the zinc of carboxypeptidase B with cobalt increases the peptidase activity of this enzyme one-fold and lowers the esterase activity under certain conditions. In contrast, replacement with cadmium completely deletes the peptidase activity and increases the esterase activity one-fold. Preliminary kinetic investigations indicate that the loss in activity in each case is due to loss in ability to hydrolyze bonds rather than loss in binding capacity.

Several synthetic peptides, in which lysine is the penultimate C-terminal residue, are slowly hydrolyzed by carboxypeptidase B at high concentrations. The slow rate of hydrolysis apparently is due to low affinity of the substrates for the enzyme.

A several-fold purification of procarboxypeptidase B has been achieved. Activation of the zymogen was unexpectedly encountered during attempts at ion exchange cellulose chromatography.

(b) Highly purified carboxypeptidase B was found to have the amino-terminal sequence, threonylseryl (aspartic acid or asparagine) and the carboxyl-terminal sequence (serine, valine) asparaginyl-threonine.

II. Peptides and derivatives of the general formula, R-argininyl-R^1 where R = glycyl, glycylglycyl, phenylalanyl, or aspartyl and R^1 = amide or glycine amide, have been prepared and studied as thrombin substrates. No outstanding differences in rates of hydrolysis are evident.

Peptides derived from thrombin, which was inactivated with P^{32} diisopropyl phosphorofluoridate, have been separated by ion exchange and peptide "mapping."

The entire amino acid sequence of peptide B has been obtained. It is extremely interesting that the N-terminal amino group of this peptide is blocked with an acetyl grouping. The N-acetyl grouping has been previously reported in Tobacco Mosaic Virus and in the Melanocyte Stimulating Hormone of pig anterior pituitary gland.

III. Peptide "maps" have demonstrated definite differences in the two genetic species of β-lactoglobulin. The previous suggestion that this protein is composed of two identical chains has been substantiated by the fact that the proper theoretical number of peptides has been separated following exhaustive digestion by trypsin.

Significance to Dental Research:

I. Knowledge of the specificity and mechanism of action of the carboxypeptidases contributes to an understanding of basic biochemical processes. Carboxypeptidase B has, furthermore, become a valuable tool in protein and peptide structure research as well as a means of inducing limited protein modification.

II. An understanding of the primary structures and basic mechanisms involved in the fibrinogen-fibrin transition should contribute to a systematic pharmacological and clinical approach to the correction of certain abnormal blood clotting reactions.

Proposed Course of Project:

I. Studies are under way to gain more insight into the active center of the carboxypeptidases, i.e. groupings to which the metal is bound, sequence of amino acids in the vicinity of the metal, kinetics of the native, cobalt and cadmium carboxypeptidases, etc.

II. Conformation of certain peptide sequences in the B peptide from fibrinogen must be obtained by comparison of natural with synthetic peptides, e.g., the N-acetyl threonylglutamic acid sequence.

III. Attempts will be made to identify the positions and areas wherein the differences in the species of β-lactoglobulin exist.

Part B included Yes

Serial No. NIDR-20

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Folk, J. E., Piez, K. A., Carroll, W. R. and Gladner, J. A. Carboxypeptidase B. IV. Purification and Characterization of the Porcine Enzyme. J. Biol. Chem. 235:2272-2277, Aug. 1960.

Folk, J. E., and Gladner, J. A. Cobalt Activation of Carboxypeptidase. A. J. Biol. Chem. 235:60-63, Jan. 1960.

Folk, J. E. and Gladner, J. A. The Amino Acid Sequence of Peptide B of Co-fibrin. Biochim. et Biophys. Acta 44:383-385, Nov. 4, 1960.

Laki, K., Gladner, J. A. and Folk, J. E. Some Aspects of the Fibrinogen-fibrin Transition, Nature, 187:758-762, Aug. 27, 1960.

Folk, J. E. and Gladner, J. A. Influence of Cobalt and Cadmium on the Peptidase and Esterase Activities of Carboxypeptidase B. Biochim. et Biophys. Acta, in press.

Folk, J. E., Braunberg, R. C., and Gladner, J. A. Carboxypeptidase B. V. Amino and Carboxyl-terminal Sequences. Biochim. et Biophys. Acta, in press.

Serial No. NIDR-21
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Biochemical and Biophysical Studies of Calcification

Principal Investigator: Dr. R. C. Likins

Other Investigators: None

Cooperating Units: National Bureau of Standards
NIAID
U. S. Geological Survey
The Roosevelt Hospital, New York

Man Years (calendar year 1960):

Total:	3
Professional:	1
Other:	2

Project Description:

Subproject A: (With Dr. A. S. Posner, National Bureau of Standards)

Objectives:

To investigate the skeletal metabolism of calcium and strontium.

Methods Employed:

The deposition of injected Sr^{89} and Ca^{45} was compared in several different calcified tissues of the rat. X-ray diffraction line broadening studies were performed on these same tissues and the corrected width at half maximum obtained for the 002 reflection.

Major Findings:

Discrimination against strontium relative to calcium increased with an increase in crystal perfection as determined by X-ray diffraction line broadening analysis. These results are in accord with a previous study where the final composition of a series of synthetic hydroxyapatites was related to the degree of crystal perfection (see Project Report for Calendar Year 1959). The results also provide evidence of a range of size and/or crystal perfection in the crystal texture of various calcified tissues with dentin lying between bone and enamel in this series. Differences also were found

in the crystal properties of such similar materials as molar dentin and incisor dentin. Indeed, the crystallinity within adjacent regions of a single tissue may differ as shown by a preliminary observation that rat incisor dentin increases in crystallinity from the proximal to the incisal end.

Significance to Dental Research:

The results provide information on factors affecting the composition and properties of calcified tissues.

Proposed Course of Project:

This work has been completed and a report is ready to submit for publication.

Subproject B: (With Dr. E. G. Berry, NIAID)

Objectives:

To investigate the comparative metabolism of calcium and strontium in calcifying aragonite.

Methods Employed:

Immature specimens of Australorbis glabratus, a freshwater pulmonate snail, were selected for study. Approximately 7 mm. of the body whorl of each snail was removed with fine dissecting forceps and the snails placed in solutions containing Ca^{45}, Sr^{89}, and various concentrations of non-radioactive calcium and strontium. After two days, samples of the newly formed shell and of old shell were removed and analyzed for radiocalcium and radiostrontium.

Major Findings:

Shell formed during the course of the experiment showed a marked discrimination against strontium. The magnitude of this discrimination increased as the concentration of calcium in solution was increased relative to that of strontium. The preferential utilization of calcium in the biological crystallization of aragonite (snail shell) is greater than in the case of hydroxyapatite (bone and teeth).

Significance to Dental Research:

The results provide information on the comparative mineral metabolism of different calcifying systems.

Proposed Course of Project:

The experiment is completed and no further extension of this work is

contemplated at this time.

Subproject C: (With Dr. M. Rubin, U. S. Geological Survey, and Dr. E. G. Berry, NIAID).

Objectives:

To determine the extent to which aquatic and land snails utilize calcium carbonate as a source of carbonate in shell formation.

Methods Employed:

Aquatic snails (A. globratus) were prepared as described in Subproject B and placed in tanks containing carbon-14 labelled calcium carbonate. After two or three days samples of new and old shell were obtained and the incorporation of carbon-14-labelled carbonate determined. Terrestrial snails (T. tridentata and T. albolabris) were prepared in the same manner and placed in a glass terrarium containing lettuce dusted with the labelled $CaC^{14}O_3$. At the end of one week samples of new and old shell were analyzed for carbon-14-labelled carbonate.

Major Findings:

In both the aquatic and terrestrial snails, approximately ten percent of the carbonate deposited in the newly formed shell had been derived from the labelled calcium carbonate. In both groups approximately one percent of the carbonate of the old shell had been replaced by carbonate derived from the labelled material.

Significance:

Pulmonate and land snails are of importance in geological dating using the C-14 method devised by Libby. Recently the use of such shells has been challenged on the grounds that snails living in a calcareous loess environment use a substantial amount of the dead carbonate of the limestone particles for the making of their shells yielding, thereby, excessively old dates. The results obtained in this study provide an estimate of the error of such age determinations.

Proposed Course of Project:

No further extension of this work is planned.

Subproject D: (With Dr. S. Natelson, The Roosevelt Hospital, New York)

Objectives:

To explore the feasibility of using a dietary calcium additive as a means of reducing the skeletal burden of strontium-90 in children.

Part A. Cont'd. Serial No. NIDR-21

Methods Employed:

Subjects will be selected from 3600 school children residing in eight boarding schools located in North Dakota and South Dakota. One half of these children are now receiving a dietary calcium supplement which has approximately doubled their calcium intake. Serum obtained from the subjects will be analyzed for calcium and for strontium by X-ray spectroscopy to determine whether or not the calcium supplement has significantly reduced the serum Ca/Sr ratio.

Major Findings:

Progress to date has been limited to the development by Dr. Natelson of a sensitive technique for determining the strontium content of serum. It is anticipated that the clinical study will be underway early in 1961.

Significance:

Concern over possible hazards associated with the ingestion of Sr^{90} has focussed attention on strontium metabolism and, particularly, on means of effecting a reduction in present and future skeletal burdens. There can be no question that a reduction in Sr^{90} deposition especially during childhood and adolescence, when skeleton accretion is most rapid, is desirable. It is reasonable to suppose that this can be accomplished by diluting Sr^{90}, ubiquitously present in dairy, grain, and other foodstuffs, with sources of dietary Ca free of this nucleide. The attendant lowering of the dietary Sr^{90}/Ca ratio would be expected to increase the competitive disadvantage of Sr^{90}, relative to Ca, resulting in a diminished absorption and retention of the former by the child.

Proposed Course of Project:

It is anticipated that the investigation will be extended to include a strontium and calcium balance study of selected control and calcium supplemented children. In addition, absolute assay of strontium^{90} in the food and excreta of these subjects will be carried out.

Part B included Yes

Serial No. NIDR-21

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Likins, R. C., Piez, K. A., and Kunde, M. L. Mineralization of the Turkey Leg Tendon. III. Chemical Nature of the Protein and Mineral Phases in "Calcification in Biological Systems," ed. by R. F. Sognnaes, Amer. Assoc. for the Advancement of Science, 1960, p. 143-149.

Piez, K. A., and Likins, R. C. The Nature of Collagen. II. Vertebrate Collagens in "Calcification in Biological Systems," by R. F. Sognnaes, Amer. Assoc. for the Advancement of Science, 1960, p. 411-420.

Likins, R. C., McCann, H. G., Posner, A. S., and Scott, D. B. Comparative Fixation of Calcium and Strontium by Synthetic Hydroxyapatite. J. Biol. Chem. 235:2152-2156, July 1960.

Likins, R. C., and Craven, D. L. An Apparent Difference in the Renal Clearance of Ca^{45} and Ca^{40} in Rats. Nature 188:592-593, Nov. 1960.

Serial No. NIDR-22
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Biochemistry and Physiology of the Salivary Glands and Their Secretions

Principal Investigator: Dr. I. Zipkin

Other Investigators: None

Cooperating Units: U.S. Naval Training Station, Great Lakes, Illinois
Laboratory of Microbiology and Clinical Investigations, NIDR

Man Years (calendar year 1960)

Total:	2½
Professional:	½
Other:	2

Project Description:

Objectives:

(1) To study basic organic components of parotid and submaxillary saliva and their relation to oral and systemic diseases.

(2) To determine to what extent constituents of the salivary secretions are a blood ultrafiltrate vs a product of intrinsic metabolism.

Subproject A:

Development of a Simplified Procedure for the Determination of Uric Acid, Protein, Tyrosine and Tryptophan in Parotid Saliva from the Ultraviolet Absorption Patterns.

Methods Employed:

Parotid saliva has been examined from individuals with periodontal disease, gout, arthritis and Sjogren's syndrome. In addition, parotid saliva has been analyzed from albinos of a racial isolate in Maryland, and from caries-free and caries-rampant individuals of the U.S. Naval Training Station, Great Lakes, Illinois.

Major Findings:

Previous data have shown correlation coefficients of 0.94 and 0.98 for tyrosine and tryptophan respectively, by chemical and spectrophotometric comparison on the TCA precipitable protein. A high correlation was also found for protein determined by chemical vs spectrophotometric procedures. These procedures were applied to whole parotid saliva. Preparation of ultraviolet absorption spectra before and after uric acid treatment have allowed calculation of uric acid, tyrosine and tryptophan from these spectra.

Total protein values by Lowry and spectrophotometry on the whole parotid saliva did not correlate.

Saliva of individuals with arthritis, Sjögren's syndrome and periodontal disease showed no variations from the normal, whereas saliva of racial isolates (Albinism) was low in tyrosine and tryptophan. The parotid saliva of gout patients was elevated in uric acid, the data correlating reasonably well with blood uric acid.

Subproject B:

Sialic Acid in Human Saliva.

Methods Employed:

Sialic acid was determined in parotid and mixed human saliva by the procedure of Warren. Its presence was confirmed by determination of the absorption spectrum of the chromophore, by the Bial and direct Ehrlich reaction and by paper chromatography.

Major Findings:

Parotid saliva contained 2.5 mg. % sialic acid whereas whole saliva contained 5.0 mg. %. Although sialic acid has been found in many tissues including the salivary glands its presence in the salivary secretions has not been reported in the literature.

Subproject C:

The calcium, phosphorus total solids and ash in whole saliva of children receiving supplements of $CaHPO_4$ in the bread flour.

Methods Employed:

Whole saliva was collected in September 1959 and again in April-May 1960 from 8-12 year children receiving $CaHPO_4$ supplements in the bread. Appropriate controls were also available. Whole

saliva contained 4.5 mg. % Ca, 12.5 mg. % P, 0.50% solids, and about 0.15% ash. These data did not correlate with age, sex or $CaHPO_4$ ingestion.

Significance to Dental Research:

Since saliva is in intimate contact with the teeth and other oral structures, it is important to study its chemistry in both normal and pathological states. Since the parotid secretion is a discreet body fluid, it is important to determine to what extent it reflects oral and systemic disease.

Proposed Course of Project:

(1) Saliva and blood will be examined for their tyrosine, tryptophan, uric acid and protein content in individuals with disease states which may lead to changes in these metabolites.

(2) Sialic acid is a constituent of most mucoproteins, mucopolysaccharides and mucolipids and hence has a ubiquitous distribution. It is a component of the "cementing substances" in soft tissues and bone. Inflamed gingival tissue and the epiphyseal plate in pathological calcifications will be examined for sialic acid. Oral bacteria will also be analyzed for sialic acid.

(3) Since the $CaHPO_4$ level in the bread-flour has been raised, one more saliva collection will be made in May 1961.

(4) In collaboration with Dr. King, the effect of various endocrinectomies on the chemistry of the salivary secretions in the rat will be studied, and in the human where possible.

(5) In collaboration with Dr. L. Avioli, studies will be conducted on the comparative concentrations of Ca^{47} and Mg^{28} in the blood and parotid saliva after intravenous adminstration of the isotope.

(6) In collaboration with Dr. King, studies will be conducted on the chemistry of parotid saliva as related to "Antarctic stress."

Part B included Yes

Serial No. NIDR-22

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Geever, E. F., McClure, F. J., and Zipkin, I.: The salivary glands in lysine-deficient rats. J. Dent. Res. 39: 129-140, Jan-Feb. 1960.

Zipkin, I., Larson, R. H., and Rall, D. D.: Reduced caries activity in offspring of rats receiving tetracycline during various prenatal and post-partum periods. Proc.Soc.Exptl.Biol. & Med. 104: 158-160, May 1960.

Zipkin, I.: The effects of the absorption of fluoride. IV. The deposition of fluoride in human skeletal tissue as related to fluoride in the drinking water. A. M. A. Arch. Ind. Health 21: 329, April 1960.

Zipkin, I.: The effects of the absorption of fluoride. VIII. The fluoride content of calculi of the urinary and biliary tracts. A. M. A. Arch. Ind. Health 21: 338-339, April 1960.

Zipkin, I., McClure, F. J., and Lee, W. A.: Relation of fluoride content of human bone to its chemical composition. Arch. Oral Biol. 2: 190-195, 1960.

Zipkin, I.: Chemical agents affecting experimental caries. J. Amer. Med. Assoc. In press.

Larson, R. H., and Zipkin, I.: Effect of tetracycline on the transmission of dental caries in rats. J. Dent. Res. In press.

Larson, R. H., Zipkin, I., and Rubin, M.: Effect of administration of EDTA by various routes on dental caries in the rat - Possible role of coprophagy. Arch. Oral Biol. In press.

Serial No. NIDR-23
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Role of Citrate in the Calcifying Tissues.

Principal Investigator: Dr. I. Zipkin

Other Investigators: None

Cooperating Units: National Cancer Institute
Pennsylvania State University

Man Years (calendar year 1960)

Total:	2
Professional:	½
Other:	1½

Project Description:

Objectives:

To elucidate the role of citrate in calcified tissues.

Subproject A.

Relation of fluoride to citrate content of bone.

Methods Employed:

It has been shown previously that the fluoride concentration of bone is inversely related to its citrate concentration. Recently, tibia-fibula preparations obtained from rats receiving 0, 10, 50 and 100 ppm fluoride in the drinking water were sacrificed at 72, 102 and 161 days of age. Tissues were analyzed for citrate by a modification of the method of Hess et al.

Major Findings:

The concentration of citrate in the tibia-fibula of rats receiving 0, 10, 50 and 100 ppm F in the drinking water at 72 days of age was 0.53%, 0.53%, 0.49% and 0.43%; at 102 days it was 0.53%, 0.45%, 0.41% and 0.36%; and at 161 days it was 0.50%, 0.46%, 0.37% and 0.37%. Hence the citrate concentration decreased consistently with increased fluoride exposure, irrespective of age. Also, irrespective of the level of fluoride administration,

the citrate content of the bone decreased with age.

Proposed Course of Project:

The effect of level of calcium nutrition on the fluoride-citrate relation will be studied. Also attempts have been made to obtain highly fluorosed cattle bones from the Utah State University study conducted by Dr. D. A. Greenwood of Utah State University. Studies will be conducted to determine if the fluoride-citrate relationship is enzymatic controlled.

Subproject B:

Citrate concentration of teeth of various species.

Methods Employed:

The molars and incisors of various species of mammal were analyzed for citrate by a modification of the method of Hess et al.

Major Findings:

Data have been accumulating in this laboratory to show that the citrate content of the teeth of various species differs considerably. Man, monkey, dog and fox contained 880, 510, 437 and 252 mg %, respectively in the molar dentin. The hamster, peromycus, cotton rat, mouse and white rat contained 125, 137, 287, 352 and 470 mg % citrate in the molar dentin respectively. The molar enamel contained 1/5 to 1/10 as much citrate. The concentration of citrate in the incisors of the rodents was considerably less than in the molars.

Significance to Dental Research:

Citrate is present in the teeth and bones in appreciable concentrations. It appears to increase in conditions favoring bone resorption, but these processes are little understood. The purpose of these studies is to help define the role of citrate in calcifying tissue.

Proposed Course of Project:

(1) In cooperation with Dr. L. Avioli, studies are in progress on the concentration of citrate in the blood and urine of patients with metastatic bone disease.

(2) In collaboration with Dr. G. Martin, the concentration of citrate in some pathological calcification such as lathyrism will be investigated.

Part B included No

Serial No.NIDR-24
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Correlation of Clinical Oral Health Findings to Biochemical Stress-related Factors in Personnel Wintering in Antarctica

Principal Investigator: Dr. C. T. G. King

Other Investigators: Dr. I. Zipkin

Cooperating Units: U. S. Naval Medical Research Laboratories, U. S. Naval Submarine Base, New London, Conn.

Man Years (calendar year 1960):

Total:	1/4
Professional:	1/4
Other:	none

Project Description:

Objectives:

During a period of stress, stimulated adrenal gland activity increases epinephrine, nor-epinephrine and adrenal-corticoid steroid levels in the blood. Personnel wintering in Antarctica are subject to emotional and physical stress due to isolation and exposure to extreme cold. A biochemical study of stress-related factors in blood, urine, and saliva should lead to some understanding of systemic manifestations of this stress environment. The objective of this study is to correlate clinical oral health findings with various biochemical-stress related factors.

Methods Employed:

A number of samples of blood, urine, and saliva have been obtained from Navy personnel prior to their departure to Antarctica and more samples are to be obtained during their stay there. The following determinations will be made:

Blood Serum: Adrenal corticoid level, calcium, sodium and potassium will be determined.

Urine: Catichol amines: Epinephrine and nor-epinephrine.

Part A. Cont'd. Serial No.NIDR-24

Saliva: (Specifically, parotid saliva) Steroids, Na, K, proteins, tyrosine, tryptophan, uric acid and total carbohydrate.

Clinical Phase: (To be carried out by Dr. T. Allensworth, U.S.N., in the Antarctica)

1. Evaluation of gingival tissue: (a) P M A index ; (b) Intra-oral colored photographs.

2. Evaluation of supporting structure of teeth: (a) Full mouth radiographic survey at six months intervals; (b) Russell's Index; (c) Tooth Movilometer.

3. Evaluation of Nutritional Status: (a) Intradermal dye-oxidation test for tissue levels of Vitamin C; (b) Wood's light illumination of tongue for fluorescent patterns and possible photographic record.

Significance to Dental Research:

As previously mentioned, it is the objective of this study to correlate clinical oral health findings with various biochemical or physiological stress related factors.

Proposed Course of Project:

At the end of the geophysical year all of the samples obtained in the Antarctica will be forwarded, in a frozen state, to the National Institute of Dental Research where the biochemical determinations will be carried out. The data will then be evaluated and correlated with Dr. Allensworth's findings before any further work is planned on this project.

Part B included No

Serial No. NIDR-25
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effect of Prenatal Factors on Fetal Development and Dental Caries in the Progeny

Principal Investigator: Dr. C. T. G. King

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):

Total:	2¼
Professional:	3/4
Other:	1½

Project Description:

Subproject A:

Dental caries in the progeny of acidotia pregnant rats.

Objectives:

To study the effect of acidosis during pregnancy on the caries susceptibility of the offspring.

Methods Employed:

Pregnant rats were rendered acidotic by exposure to an atmosphere of 30% carbon dioxide in 70% oxygen at different periods of gestation. Litters from treated and non-treated females were reduced to six in number and after 21 days of lactation were fed a cariogenic skim milk powder diet. After a period of 65 ± 5 days the rats were killed and the teeth diagnosed for caries. Blood serum was obtained for calcium and phosphorus determination and liver, muscle, and adrenal specimens were obtained for future glycogen analysis.

Major Findings:

There were 75 rats whose mothers had been exposed to the CO_2 atmosphere during the 7th day of gestation for 30 minutes, and on the 8th and 9th day for 10 minutes. 97.4% were carious and has an average severity score of 19 ± 1.8. Males had a lower

caries score (15.22 ± 1.8) than females who had a score of 22.6 ± .96. Among 67 rats from non-treated females, 83.6% developed caries with an average score of 9.25 ± 1.23, about half that of the treated group. Acidosis after the 14th day of pregnancy did not alter the caries susceptibility of the offspring. At no period of gestation did this treatment induce caries in the offspring maintained on a normal stock diet.

Significance to Dental Research:

This project attempts to evaluate the effect of pre-natal influences on the development of dental caries.

Proposed Course of Project:

This experiment is being repeated to confirm the initial findings. An additional experiment is in progress in which the mothers were stressed on the 7th, 8th and 9th day of pregnancy with one of the following agents.

(a) Hydrocortisone
(b) Tremorine
(c) Electroshock
(d) Anemia (by repeated cardiac punctures and blood drawings)

Subproject B:

Prenatal Influences on Fetal Development.

Objective:

To study the relation of variations in amniotic pressure on the development of the rat fetus.

Methods Employed:

Amniotic pressure was reduced by withdrawing 0.10 cc of amniotic fluid from the sack, and was increased by transferring this fluid to the fetus near the ovary or the cervical canal on the opposite uterine horn. The injected fluid was prevented from escaping by tying off the hole made by the needle (either at the cervix or the fimbreated funnel). These manipulations were carried out during the 14th day of pregnancy. The rats were killed on the 20th day and the fetuses examined grossly for malformations.

Major Findings:

(1) Palatal development was affected by both decreasing and increasing amniotic pressure. The following results were obtained

(a) among 97 fetuses which developed while the amniotic sack pressure was reduced, 6.5% had cleft palates, 9.2% had limb deformities, and there were 49% resorptions; (b) Among 94 fetuses developed under increased sack pressure, 14.9% had cleft palates, 4.5% had limb deformities, 3.5% had umbilical cord hernia and this procedure produced 32% resorptions. A total of 487 normal fetuses were examined and only one had a cleft palate.

Significance to Dental Research:

This study was carried out to evaluate some of the factors which may contribute to congenital abnormalities in the oral structures.

Proposed Course of Project:

A number of chemotherapeutic agents will be screened for their teratological effects. All of the fetuses will be cleared and stained with alizarin red to determine if skeletal defects, in addition to cleft palate, are produced.

Part B included No

Serial No. NIDR-26
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies on Ascorbic Acid. I. The Transport of Ascorbic Acid. II. Role of Ascorbic Acid in Formation and Maintenance of Dental Structures.

Principal Investigator: Dr. G. R. Martin (ADA Research Associate)

Other Investigators: Dr. H. M. Fullmer

Cooperating Units: American Dental Association

Man Years (Calendar year 1960):

Total:	1
Professional:	½
Other:	½

Project Description:

Objectives:

I. To determine the mode of transport of ascorbic acid into cells.

II. To evaluate the role of ascorbic acid and other compounds in the formation and maintenance of dental structures.

Methods Employed:

Standard chemical methods for estimating ascorbic acid were employed. Radioactive determinations were carried out by a carrier dilution procedure.

Major Findings:

(a) It was found that L-ascorbic acid-1-C^{14} slowly equilibrates in various tissues when injected into rats, whereas dehydroascorbic acid equilibrates more rapidly. Dehydroascorbic acid was found to penetrate into brain, eye and erythrocytes to a greater extent than ascorbic acid. Intravenous administration of large doses of ascorbic acid to nephrectomized rats or the induction of accelerated ascorbic acid biosynthesis in such animals resulted in a marked accumulation of the vitamin in serum without appreciable tissue transfer. A similar amount of exogenous dehydroascorbic acid, the oxidized non-ionic form of the vitamin, was largely removed from serum. The ascorbic acid in serum of blood drawn from the renal

vein of dogs was primarily in an oxidized form, whereas ascorbic acid in serum from the carotid artery, femoral and jugular veins was largely in the reduced form. These findings suggest that ascorbic acid enters tissues as dehydroascorbic acid and that the kidney may play a role in the distribution of the vitamin by oxidizing it to the unionized easily penetrable form.

(b) Since ascorbic acid is essential for dentinogenesis, dental structures are particularly sensitive to ascorbic acid deficiency. Using dental structures for an assay system, studies on scorbutic guinea pigs treated with 3-methylcholanthrene, D-ascorbic acid and 2,6 dichlorophenolindolphenol indicate that each of these may prevent some but not all of the pathoses of scurvy. These observations suggest that vitamin C has multiple functions.

(c) Microscopic examination of scorbutic guinea pig jaws indicates that granules of enamel matrix accumulate in and around ameloblasts situated adjacent to the site which experienced a failure of dentinogenesis. This finding indicates that the normal progress of amelogenesis requires the presence of normal dentin. The degeneration of odontoblasts in ascorbic acid deficiency appears to be a fundamental cause of the gross changes seen in dental structures.

Significance to Dental Research:

Considerable epidemiological evidence indicates that ascorbic acid deficiency is a factor in various human oral diseases. A precise understanding of the metabolism and function of ascorbic acid in the body may increase our understanding of various oral diseases.

Proposed Course of Project:

I. Further studies are in progress to evaluate the intestinal absorption of ascorbic acid and the manner in which it is stored in various tissues.

II. Biochemical studies will investigate the manner in which 3-methylcholanthrene reverses the symptoms of scurvy.

Part B included Yes

Serial No. NIDR-26

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Martin, G. R., and Mecca, Christyna E. Studies on the Distribution of L-ascorbic Acid in the Rat. Arch. Biochem. Biophys. In Press.

Martin, G. R. Studies on the Tissue Distribution of Ascorbic Acid. Annals New York Acad. Science. In Press.

Martin, G. R., Fullmer, H. M., and Burns, J. J. Observations on the effect of 3-methylcholanthrene in scorbutic guinea pigs. Proc. Soc. Exptl. Biol. Med. In Press.

Burns, J. J., Conney, A. H., Dayton, P. G., Evans, C., Martin, G. R., and Taller, D. Observations on drug induced synthesis of D-glucuronic, L-gulonic and L-ascorbic acid in rats. J. Pharm. & Exptl. Ther. 129:2:132-138, June 1960.

Fullmer, H. M., Martin, G. R., and Burns, J. J. Role of ascorbic acid in the formation and maintenance of dental structures. Annals New York Acad. Science. In Press.

Serial No. NIDR-27
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Relation of Dietary Minerals and Protein to Dental Caries

Principal Investigator: Dr. F. J. McClure

Other Investigators: Dr. I. Zipkin, Dr. E. F. Geever

Cooperating Units: None

Man Years (calendar year 1960):

Total:	4½
Professional:	1
Other:	3½

Project Description:

Objectives:

To obtain information on the effect of calcium and phosphate minerals and cereal proteins on experimental rat caries.

Methods Employed:

Different common cereal foods and their commercial counterparts, particularly breakfast foods, are incorporated into experimental diets for white rats. Special phosphate minerals are used in different cariogenic test diets to determine their anticaries effect and their mode of action.

Major Findings:

1. Additional data were obtained on the cariostatic effect of phosphates added to baking flours and bread. An unexpected and significantly cariostatic effect of sodium phytate was reported. This insoluble phosphate would appear to become active in the oral cavity only through hydrolysis by phytase.

2. Drastically altered calcium phosphorus ratios of the diet changed the mineral composition of certain calcified tissues including the third molar and incisor teeth of white rats, but these changes had no relation to the dental caries experience.

3. A study on young white rats indicated that a severe deficiency in the essential amino acid, lysine, impaired the development of the salivary glands and the tongue. In general, other than characteristic effects on growth, there were no specific effects of a lysine deficiency per se. Previously we identified lysine as a corrective of the cariogenic effect of heat-processed foods.

4. Pronounced experimental dental caries resulted on feeding a diet containing essentially millet and no free sugar. There is continuing evidence that dental caries may be produced experimentally by an ever increasing number and variety of diets, particularly without an excessive quantity of free sugar or coarse food particles. The mechanism by which these various diets produce caries may encompass a number of different processes.

5. A diet consisting mainly of ground whole oats, caused a rapid and extensive loss of gingival tissue and alveolar bone in white rats. Presumably this was due to impaction of fragments of oat hulls, food and hair.

Significance to Dental Research:

Evidence obtained through experimental animal studies continues to indicate that phosphates have an unusual cariostatic effect. Although the mechanism of this action is unresolved, there remains the possibility of utilizing this phosphate effect for human caries control. Attempts to relate the calcium and phosphorus content of the diet to the chemistry of the teeth and dental caries have generally failed. Thus there is increasing evidence that adequate provision of calcifying factors of the diet offers no solution for the control of dental caries. There is continuing interest concerning the relation of cereal foods and inadequate dietary protein to dental caries etiology.

Proposed Course of Action:

Continuing experimental studies to evaluate the mechanism of the phosphate anticaries effect, and exploration of the cariostatic effect of different calcium minerals.

Part B included Yes

Serial No. NIDR-27

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

McClure, F. J. The Cariostatic Effect in White Rats of Phosphorus and Calcium Supplements Added to the Flour of Bread Formulas and to Bread Diets. J. Nutrition 72:131-136, Oct. 1960.

McClure, F. J. and McCann, H. G. Dental Caries and the Composition of Bones and Teeth of White Rats as Affected by Variations in Dietary Ash, Calcium and Phosphorus. Arch. of Oral Biol. 2:151-161, July 1960.

McClure, F. J. Millet (Cattail and Foxtail) as the Cariogenic Component of Experimental Rat Diets. J. Dent. Res. In press.

McClure, F. J. Alveolar Bone Resorption in White Rats Fed a Diet Consisting Predominantly of Whole Oat Flour. J. Dent. Res. In press.

Geever, E. F., McClure, F. J. and Zipkin, I. The Salivary Glands in Lysine-deficient Young Rats. J. Dent. Res. 39:129-140, Jan.-Feb. 1960.

Serial No. NIDR-28
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Role of Bacteriology and Nutrition in Experimental Dental Caries

Principal Investigator: Dr. R. H. Larson

Other Investigators: Dr. R. J. Fitzgerald, Dr. I. Zipkin

Cooperating Units: Gnotobiotic Section
Laboratory of Microbiology, NIDR

Man Years (Calendar Year 1960):

Total:	2
Professional:	1
Other:	1

Project Description:

Subproject A:

Experimental procedures essential for the study of stabilized oral flora in relation to experimental dental caries.

Objectives:

To define the condition required to study independently the effects of microbial flora vs. nutritional variables on experimental dental caries.

Methods Employed:

Conventional animal experimental-regimens have been utilized to evaluate caries activity in relation to variations in pre-treatment with broad-spectrum antibiotics; i.e. age of animal, source of inoculum, method of inoculation, nutrition of animal and toxicity.

Major Findings:

With adequate care rats can be weaned as early as the 16th day making it possible to control the total nutrition at an earlier age than assumed possible heretofore.

Once an animal has established its flora it appears difficult for new organisms to be established. The most effective means of establishing a desired flora was by depression of the normal flora with a broad-spectrum antibiotic followed by an inoculum of the desired flora.

The period of antibiotic treatment necessary to depress caries active flora may be as short as three days. The age of the animal at the time the antibiotic is administered is also extremely important. It appears that smooth surfaces can be reduced at any age, but if the caries active flora have become established in the "hard to reach" sulci before the antibiotic treatment period, only minimum reduction of the sulcal caries can be obtained. The antibiotic treatment appears to be more effective if completed before the rat is three weeks old. Several methods have been used to introduce a caries active flora into the rat's oral cavity. The introduction of a tagged (antibiotic resistant) organism in pure culture, which was caries active in the gnotobiotic rat, caused no increase in caries and was not recovered in pure culture. This finding indicates that an organism which may be quite active under one set of circumstances (gnotobiotic animal) may not become established under other circumstances (conventional animal).

Thus far the most effective introduction of a caries active flora was accomplished by housing "depressed flora" animals with caries-active animals at the beginning of the caries test period. As inoculum, fresh or lyophylized feces or cecum contents have brought about only slight increases in caries activity. Four groups of rats with different degrees of caries activity were used to inoculate four groups of young antibiotic treated animals. The caries activity of the young rats was of the same order of magnitude as the animals used for inoculum. Apparently there are qualitative or quantitative differences in the transmissible flora of various groups of caries-active animals.

Significance to Dental Research:

In most experimental rat caries studies dietary variations are introduced either prior to or during a caries-test period. In these studies generally no attempt has been made to equalize the initial oral flora of the various groups. Numerous dietary regimens have been associated with an increase in caries activity. It has not been known, however, that this was because the teeth became more susceptible under the dietary regimen or that the diet established a more active oral flora. If the actual causative factors associated with experimental caries activity may be determined, the results will have increased value as applicable to the etiology of human caries.

Part A. Cont'd. Serial No. NIDR-28

Proposed Course of Project:

To advance this research it is essential to have available a standardized caries active flora which can be easily introduced into the oral cavity. It is desirable that the organisms be grown in pure culture.

The selection of a caries test-diet is important and it is our aim to formulate a synthetic diet which is nutritionally adequate for the rat and will also support the caries process.

Part B included Yes

Serial No. NIDR-28

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Zipkin, I., Larson, R. H., and Rall, D. P. Reduced Caries Activity in Offspring of Rats Receiving Tetracycline during Various Prenatal and Post-partum Periods. Proc. Soc. Exp. Bio. & Med. 104:158-160, May 1960.

Larson, R. H., and Zipkin, I. Effect of Tetracycline on the Transmission of Dental Caries in Rats. J. Dent. Res. In Press.

Larson, R. H., Zipkin, I., and Rubin, M. Effect of Administration of EDTA by Various Routes on Dental Caries in the Rat - Possible Role of Coprophagy. Arch. Oral Biol. In Press.

Serial No. NIDR-29
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Analytical and Structural Aspects of Collagen and Proteins of Related Interest in Relation to Function.

Principal Investigator: Dr. K. A. Piez

Other Investigator: Dr. M. S. Lewis

Cooperating Units: National Institute of Arthritis and Metabolic Diseases
Western Reserve University
National Bureau of Standards
Massachusetts General Hospital
University of Kansas City

Man Years (calendar year 1960):

Total:	1½
Professional:	½
Other:	1

Project Description:

Objectives:

It is the long-range purpose of this project to examine collagens and other proteins of related interest with respect to their chemical and physical nature and possibly correlate function with structure in normal and disease states. The immediate objectives relate to (1) separation and analysis of the component parts (chains) of the collagen molecule, (2) analysis of collagens from different tissues, (3) analysis of thyroglobulin, (4) analysis of carboxypeptidase B, (5) a comparison of two genetically different β-lactoglobulins, and (6) analysis of enamel protein.

Methods Employed:

Usual laboratory methods were employed.

Major Findings:

(1) Denaturation of collagen in solution by heat or urea results in the formation of at least two subfractions which are not identical. These fractions, designated α and β, have been separated by chromatography and analyzed. To improve the separation procedure

and perhaps effect resolution of still other components, newly developed procedures are being applied. This work is in progress. Dr. Lewis is collaborating in these studies.

(2) The various tissues of the eye provide a source of collagens which may show differences related to the cell of origin or to its function. Collagen is the major protein of vitrosin (a fibrillar element in the vitreous body), lens capsule, cornea, and sclera. Collagen samples have been obtained from these tissues in the calf, purified and are being analyzed. Dr. J. Gross (Massachusetts General Hospital) is performing some of the analyses.

(3) Highly purified thyroglobulin, prepared by Dr. H. Edelhoch (NIAMD), has been analyzed for its amino acid composition.

(4) Carboxypeptidase B, prepared by Drs. J. E. Folk and J. A. Gladner, was analyzed for its amino acid composition.

(5) The milk protein, β-lactoglobulin exists normally in two different genetic forms. These have been separated by Dr. E. Davie (Western Reserve University) and have been analyzed. It was found that the two forms differ in two amino acid residues. An aspartic acid and a valine in one is replaced by a glycine and an alanine in the other. The data indicate that the molecule of each consists of two identical subunits. Enzymatic degradation of the two proteins and separation of the peptides is in progress (with Drs. Folk and Gladner) in an attempt to locate and characterize the altered position exactly.

(6) Protein from the enamel of pig embryos, isolated by Dr. W. Lefkowitz (University of Kansas City Dental School), has been analyzed for its amino acid content and subjected to X-ray diffraction (by Dr. A. Posner of the National Bureau of Standards). It appears to have the crystallographic structure of a keratin but differs from other keratins in its amino acid composition. It contains a very high content of proline, and histidine is the major basic amino acid. A soluble and an insoluble protein fraction were obtained.

Significance to Dental Research:

Collagen is a major structural protein and may play a more specific role in certain processes (such as calcification). Basic studies of this protein may, therefore, contribute to a better understanding of function. The same significance, of course, attaches to all proteins. What is learned about any one protein is applicable in a general sense to all. These studies are no less important to dental research than any other research area.

Part A Cont'd. Serial No. NIDR-29

Proposed Course of Project:

Studies on the denaturation of collagen and the nature of its subunits will continue along the lines already established. The analysis of collagens from the eye, carboxypeptidase B, and of thyroglobulin are complete or will be very shortly. The two genetically different β-lactoglobulins will be studied further in an attempt to locate the genetic change. Studies to characterize enamel protein further are planned.

Part B included Yes

Serial No. NIDR-29

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Piez, K. A.: The relation between amino acid composition and denaturation of vertebrate collagen. J. Am. Chem. Soc. 82: 247 Jan. 1960.

Piez, K. A., and Gross, J.: The amino acid composition of some fish collagens: The relation between composition and structure. J. Biol. Chem. 235: 995-998, April 1960.

Likins, R. C., Piez, K. A., and Kunde, M. L.: Mineralization of the turkey leg tendon. III. Chemical nature of the protein and mineral phases in "Calcification in Biological Systems," ed. by R. F. Sognnaes, Amer. Assoc. for the Advancement of Science, 1960, pp. 143-149.

Gross, J., and Piez, K. A.: Nature of collagen. I. Invertebrate collagens in "Calcification in Biological Systems," ed. by R. F. Sognnaes, Amer. Assoc. for the Advancement of Science, 1960, pp. 395-409.

Piez, K. A., and Likins, R. C.: The nature of collagen. II. Vertebrate collagens in "Calcification in Biological Systems," ed. by R. F. Sognnaes, Amer. Assoc. for the Advancement of Science, 1960, pp. 411-420.

Folk, J. E., Piez, K. A., Carroll, W. R., and Gladner, J. A.: Carboxypeptidase B. IV. Purification and properties of the porcine enzyme. J. Biol. Chem. 235: 2272-2277, Aug. 1960.

Piez, K. A., and Morris, L.: A modified procedure for the automatic analysis of amino acids. Anal. Biochem. 1: 187-201, Nov. 1960.

Piez, K. A., Weiss, E., and Lewis, M. S.: The separation and characterization of the α- and β-components of collagen. J. Biol. Chem. 235: 1987-1991, July 1960.

NATIONAL INSTITUTE OF DENTAL RESEARCH

Individual Project Reports
Calendar Year 1960

HISTOLOGY & PATHOLOGY:

30. D. B. Scott: Studies of the Structural, Physical, and Chemical Properties of Calcified Tissues by Electron Microscopy and Electron Diffraction.

31. M. U. Nylen: Studies of the Development of Calcified Tissues Under Normal and Pathologic Conditions.

32. D. B. Scott and M. U. Nylen: Secondary Collaborative Projects and Training Activities.

33. J. G. Helmcke: Microscopic Studies of Special Structures in Dental Enamel.

34. P. H. Keyes: Experimental Dental Caries.

35. J. Theilade: Electron Microscopic Studies of Dental Calculus.

36. H. M. Fullmer: Histochemical Studies of Connective Tissue.

37. M. S. Burstone: Development and Application of Histochemical Methods in the Study of Hard and Soft Tissues.

Serial No. NIDR-30
1. Histology & Pathology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies of the Structural, Physical, and Chemical Properties of Calcified Tissues by Electron Microscopy and Electron Diffraction.

Principal Investigator: Dr. D. B. Scott

Other Investigators: Dr. M. U. Nylen

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2
Professional: 3/4
Other: 1 1/4

Project Description:

Objectives:

1. Determination of the crystal structure of enamel by electron microscopy and electron diffraction.
2. Systematic investigation of the changes in tooth surfaces occurring with advancing age.
3. Investigation of the reaction of fluoride compounds on tooth surfaces.

Methods Employed:

Negative collodion replicas and positive collodion-carbon replicas and pseudoreplicas are made from the outer surfaces of teeth and from the surfaces of ground sections. The specimens are pretreated in various fashions including simple polishing, acid etching and removal of organic components by treatment with suitable solvents. Replicas are examined under the light microscope or the electron microscope and certain preparations are in addition subjected to electron diffraction.

Major Findings:

1. Throughout the past few years studies carried on in various laboratories have led to divergent opinions on the ultimate structure of the crystallites which make up the major body of tooth enamel. Estimations have ranged from a few hundred angstroms to several thousand for the long dimension or c axis of the crystals. Previous studies in this laboratory in which crystals have been brought out either through acid action or through removal of the organic matrix with solvents such as ethylene diamine have led to the idea that the basic particle size in mature enamel may in reality fall in the range of 300A, and that these particles are aligned in a linear fashion controlled by the configurations of the underlying organic matrix. Progress made this year suggests that the long crystal-like objects which have been seen may be due to the fusion of adjacent particles which grow from nuclei seeded at approximately 300A intervals. This means then that although the individual elements within the ribbon-like configurations are on the order of 300A in size, the effective so-called crystal lengths may well reach several thousand angstroms, separation from contiguous ribbons being determined by the presence of somewhat greater quantities of intervening organic material. If this conception is correct, organic matter would then have to be considered both as occurring between crystals and within them.

2. Some years ago rather extensive studies of age changes in tooth surfaces were carried on in this laboratory. These have now been extended in order to provide further background both for studies on reactions of chemical compounds with the outer enamel surface and in connection with the development of methods for estimating chronological age of individuals for medico-legal purposes. The earlier observations that the wealth of surface structure present on the enamel when the tooth erupts wears away at a rate which depends on the location of the surface on the tooth and the position of the tooth in the dental arch has been confirmed. Surface areas have now been carefully mapped and the age changes have been plotted according to specific regions. Preliminary regression lines have been worked out for age changes on surfaces as related to chronological age of the donor of the tooth, these base-line data having been derived from several hundred teeth of known age. The first trials of these lines against "unknown" specimens from a test sample of several hundred teeth indicate that it will be possible to work out a system which is at least as accurate as the Gustafson method and which has as its main advantage the fact that the specimens do not have to be damaged. The availability of such

a method of estimating age will be of considerable value both in medico-legal science and in anthropology and it appears that the accuracy limits will fall within ± 5 years for roughly 60% of all cases and ± 10 years for roughly 80%.

3. Preceding work on the action of stannous fluoride on enamel surfaces has indicated that the pronounced protection against acid etching afforded is due mainly to the production of a surface layer of hydrous hydrated stannous oxide. It was found that this layer is highly resistant to water and acid solution and only moderately resistant to the action of abrasives. The studies have now been extended to include dentifrices containing stannous fluoride. It has been found that if teeth are suspended in the dentifrice for short periods of time marked resistance to acid action is again imparted to the surfaces but if the dentifrice is brushed on for even longer periods of time very little protection results.

Significance to Dental Research:

These continuing experiments are directed toward developing information needed for an understanding of the normal composition of enamel and its reaction to external, physical, and chemical influences.

Proposed Course of Project:

Continuation along the same lines and correlation with embryological studies.

Part B. included: Yes

Serial No. NIDR-31
1. Histology & Pathology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies of the Development of Calcified Tissues Under Normal and Pathologic Conditions.

Principal Investigator: Dr. M. U. Nylen

Other Investigators: Dr. D. B. Scott

Cooperating Units: None

Man Years (calendar year 1960):
Total: 2
Professional: 3/4
Other: 1 1/4

Project Description:

Objectives:

1. Study of the effect of a rachitogenic diet on tooth development.

2. Continued investigation into the formation of normal enamel including the submicroscopic morphology of the cells of the enamel organ.

Methods Employed:

Female rats were placed on a D-deficient diet with a high calcium and low phosphorus content (Ca:P-4-5:1) 3 weeks prior to mating in order to deplete them of vitamin D. They were maintained on the same diet during pregnancy and lactation. The offspring and normal controls were killed when four or eight days old, and the specimens fixed, dehydrated and embedded for electron microscopy and X-ray microscopy. Thin sections were cut on a Porter microtome and an LKB ultratome, while thicker sections for X-ray microscopy were cut on a modified Spencer microtome from identical blocks. To permit studies of the submicroscopic morphology of the cells of the enamel organ during progressive stages of normal enamel development the mineral was removed by placing the methacrylate embedded specimens in hydrochloric acid.

Major Findings:

1. The effect of a rachitogenic diet on tooth development.

Macroscopic Changes

The 4 and 8 day old offspring of female rats fed a rachitogenic diet (Ca:P-4-5:1) showed evidence of gross changes. Compared with the controls the animals were much smaller, and in the 8 day olds bowing of the legs was observed, indicating that the animals were suffering from rickets.

Microscopic Changes

Dentinogenesis: Dentin formation began in a normal fashion. The mesenchymal cells of the dental papilla differentiated into tall columnar cells, the odontoblasts, and with the progression of differentiation the organelles became highly organized. Paralleling this development collagen fibrils appeared at the distal ends of the cells, and bundles of thicker fibrils the (von Korff's fibers) were present between the cells. As the band of collagen matrix widened the odontoblasts withdrew leaving behind a single cellular process. Mineralization of this dentin matrix, however, did not take place at the usual level of development. Instead the band of dentin matrix continued to increase in thickness although at a much lower rate. At the present point of the investigation evidence of the presence of crystalline apatite has not been found. These observations are in agreement with previous investigations which have shown a defective mineralization of both predentin, cementum and osteoid in rachitic animals.

Amelogenesis: The most pronounced changes appeared in the ameloblasts. The cuboidal cells of the inner enamel epithelium differentiated normally into tall, columnar ameloblasts, but changes were observed both in the interrelationship between the individual cells and in the amount and distribution of the organelles. Instead of the normal close relationship between the ameloblasts, large intercellular spaces were present and in many instances the larger spaces seemed to result from degeneration of single cells. Inside the more intact cells the cytoplasm distal to the nucleus was largely occupied by numerous vacuoles which might represent a randomized type of endoplasmic reticulum since RNA granules were observed attached to their limiting membranes. The highly organized endoplasmic reticulum of the normal cell and its abundant Golgi material was not seen. The only organelles to appear normal were the mitochondria. First observations showed that in spite of the abnormal appearance of the ameloblasts enamel matrix was definitely laid

down, but at the present stage of the investigation it cannot be determined if this matrix is deposited in the usual rhythmic pattern or if it mineralizes in a normal manner. It seems evident, however, that when the deficiency is transmitted from the mother more severe changes can be created than when the deficiency is introduced directly, possibly due to the total lack of a vitamin D reserve in such experimental animals.

2. During early stages of normal ameloblast differentiation the stratum intermedium consisted of two to three layers of cuboidal cells which were in close contact with each other and with the ameloblasts. Desmosomes were observed at all points of contact between the cells. As enamel matrix formation began and progressed the stratum intermedium was reduced to a single layer of cells. These cells were scale-like with large intercellular spaces between them. Numerous finger-like projections, however, extended across the intercellular spaces, and where they touched desmosomes were present. In addition to the traditional cytologic features which did not change significantly during tooth development tonofibrils were seen in the later stages. These tonofibrils paralleled the surface of the tooth extending from the thickened cell membranes of the desmosomes toward the centers of the cells. Concomitant with these changes the stellate reticulum was reduced. In some areas both this layer and the outer enamel epithelium were missing, bringing the stratum intermedium in direct contact with the surrounding connective tissue. Capillaries were usually located in such regions next to the stratum intermedium. Between capillaries both stellate reticulum and outer enamel epithelium could be observed. In all instances from the earliest point of development a 300A thick basement membrane separated the epithelium from the connective tissue.

Significance to Dental Research:

A knowledge of the cellular processes involved in normal tooth formation is necessary in order to understand the changes which may result from systemic disorders during development. Reversely, by studying the relationships between pathological cell changes and defects in either matrix formation or mineralization an understanding of these processes may be enhanced adding to a wider knowledge of cell function in general.

Proposed Course of Project:

The investigation of normal tooth development will be continued with special emphasis on the formation of the enamel cuticle and

Part A. (Continued) Serial No. NIDR-31

the following degeneration of the cells of the enamel organ. It is intended to expand the study on pathologic tooth development by including teeth from animals fed a vitamin D free, non-rachitogenic diet (Ca:P-1,2:1). Furthermore, it is proposed to inject the animals presently studied with vitamin D and/or Phosphorus in order to study the changes taking place during recovery.

Part B. included: Yes

Serial No. NIDR-31
1. Histology & Pathology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

Nylen, M.U. and Scott, D.B. Electron Microscopic Studies of Odontogenesis. (In press, J. Ind. Dent. Assoc.)

Nylen, M.U. and Scott, D.B. Basic Studies in Calcification. J. Dent. Med. 15:80-84, 1960.

Honors and Awards relating to this project:

None

Serial No. NIDR-32
1. Histology & Pathology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Secondary Collaborative Projects and Training Activities.

Principal Investigators: Dr. D. B. Scott
Dr. M. U. Nylen

Other Investigators: None

Cooperating Units: see below

Man Years (calendar year 1960):
Total: 3
Professional: 1/2
Other: 2 1/2

Project Description:

Objectives, Methods Employed and Major Findings:

During the present year a relatively large number of requests has been received for applications of biophysical methods to problems under investigation in other laboratories. It has long been accepted that such operations as electron microscopy cannot be adapted to routine service function, primarily because each type of specimen to be studied presents new and usually difficult problems in handling, and because the microscopic observations must be made by an experienced professional investigator. Inasmuch as many of the requests represent worthwhile problems, and since most relate in some fashion to the general program of this laboratory, the staff has undertaken to offer as much secondary collaboration as seems feasible. The magnitude and success of these projects, listed below, have reached a level at which it is now necessary to consider this activity as a regular part of the Laboratory program.

1. Studies of calculogenesis; primarily electron microscopy and diffraction of intraoral deposits formed in vivo on polyester strips attached to teeth. With Dr. H. A. Zander, Eastman Dental Dispensary, Rochester, New York. To be continued.

2. Study of dental calculus isolated from antibiotic-treated and germ-free rats; electron microscopy, and electron and X-ray diffraction of scrapings, suspensions and thin sections. With Dr. R. J. Fitzgerald, Laboratory of Microbiology, NIDR. To be continued.

3. Mineralization of collagen in peritoneal transplants; electron diffraction and X-ray diffraction of intact or reconstituted collagen implanted intraperitoneally in animals; particle suspensions or sectioned material. With Dr. S. E. Mergenhagen, Laboratory of Microbiology, NIDR and Dr. G. Martin, Laboratory of Biochemistry, NIDR. To be continued.

4. Study of the calcareous corpuscles in tapeworms; electron microscopy, X-ray diffraction, electron diffraction and microradiography of isolated corpuscles and sectioned specimens, for purposes of determining composition and morphological characteristics. With Dr. T. vonBrand, Laboratory of Tropical Diseases, NIAID. To be continued.

5. Study of demineralization at neutral pH in molluscs by glandular action of the bivalve boring organ; electron microscopy, optical microscopy and electron diffraction of replicas and pseudoreplicas. With Dr. M. R. Carricker, University of North Carolina. To be continued.

6. Measurement of dimensions and demonstration of morphological outlines of apatite crystals which show chemical reactivity that varies with size; electron microscopy. With Dr. R. C. Likins, Laboratory of Biochemistry, NIDR. Completed.

7. Studies of the ultrastructure of chemically defined cellular components; electron microscopy of isolated mitochondria, RNA, and other fractions. With Dr. D. Steinberg and Dr. P. Goldman, NHI. To be continued.

8. Investigation of chromosomal ultrastructure in cultured cells; development of techniques for sectioning single cell layers and observing chromosomes electron microscopically. With Dr. R. Krooth, NIAID. To be continued.

9. Study of the effects of omission and subsequent repletion of a single essential amino-acid on the ultrastructure of tissue culture cells; electron microscopy of sectioned pellets of cells centrifuged from suspensions. With Dr. E. B. Cohen, Laboratory of Infectious Diseases, NIAID. Completed.

10. Investigation of the status of preservation of collagen fibrils after freeze-drying and rehydration of fascia prepared and banked for surgical procedures; electron microscopy of sectioned material. With Dr. R. Gresham, Tissue Bank, NMRINNMC. To be continued.

11. Development of methods for embedding and sectioning microorganisms for the observation of particle adhesion to cell membranes. With Dr. R. Cole, Laboratory of Infectious Diseases, NIAID. To be continued.

12. Estimation of sample purity and structural characteristics of isolated enamel protein and various collagens; electron microscopy of particle suspensions. With Dr. K. Piez, Laboratory of Biochemistry, NIDR. To be continued.

In addition to this collaborative work, personnel of this Laboratory were called upon to provide training of various types for a number of individuals (all Guest Workers):

1. Dr. E. P. Cohen, Laboratory of Infectious Diseases, NIAID. Training in all aspects of electron microscopy, including tissue preparation, sectioning, and care and use of the electron microscope. By agreement with Dr. Eagle, the above training is provided by this Laboratory and an electron microscope is made available by NIAMD. Period of training: one year, ended 7/1/60.

2. Dr. J. B. Theilade, Eastman Dental Dispensary, Rochester, New York. Six weeks' training in embedding and sectioning of tissues for electron microscopy.

3. Dr. Arne Bjorndal, School of Dentistry, University of Iowa. Three months' training in all aspects of specimen preparation and in electron microscopy.

4. Dr. J. A. Cran, University of Adelaide, Adelaide, Australia. Originally scheduled for three months in all aspects of electron microscopy. Dr. Cran was called back to Australia due to family reasons after the first two weeks of his stay. Arrangements are being made for him to return.

5. Dr. J. C. Parikh, School of Dentistry, Georgetown University. Intermittent periods for several months for the purpose of learning techniques of microradiography.

6. Dr. S. Takumya, College of Dentistry, New York University. Three weeks' training in the embedding and sectioning of tissues for electron microscopy.

Significance to Dental Research:

The importance of making collaborative effort available is self-evident, as is the benefit derived by those receiving training. In addition, it is felt that opportunities are provided to the staff of our Laboratory, professional and technical, both for broadening their experience in the general field of biophysical instrumentation and for contributing useful information related in most part to our interests in the oral tissues. It should be of particular interest that many of the collaborative projects are directed to various facets of the general program on studies of the mechanisms involved in mineralization.

Proposed Course of Project:

As indicated above collaborative work will continue on several problems. However, due to lack of personnel it will be necessary to effect a relatively sharp reduction in the number of joint projects which can be undertaken. If this program is to be continued and any attempt made at meeting the increasing number of requests it is urgent that at least one additional staff member be assigned to the project. The type of individual required is most likely an intermediate level professional, with a working knowledge of histology, and, if possible, electron microscopy. We feel that real opportunities for development exist for a young worker, not yet established to the point of specialized interest, who would profit by the varied associations offered.

Part B. included: Yes

Serial No. NIDR-32
1. Histology & Pathology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

Fitzgerald, R.J., Jordan, H.V., Scott, D.B., and McCann, H.G. Dental Calculus and Antibiotics in the Rat. Arch. Oral Biol. 2:85-6, 1960.

Zander, H.A., Hazen, S.P., and Scott, D.B. Mineralization of Dental Calculus. Proc. Soc. Exp. Biol. & Med. 103:257-60, 1960.

Mergenhagen, S.E., Scott, D.B., and Scherp, H. Lysis by Human Oral Bacteria of Collagen Altered by Ethylene Oxide. Proc. Soc. Exp. Biol. & Med. 103:227-9, 1960.

Martin, G.R., Mergenhagen, S.E., and Scott, D.B. Relation of Ionizing Groups to the Structure of the Collagen Polymer. (in press)

Mergenhagen, S.E., Martin, G.R., Rizzo, A.A., Wright, D.N., and Scott, D.B. In Vivo Calcification of Implanted Collagen. Biochim. et Biophys. Acta 43:562-3, 1960.

Likins, R.C., Posner, A., McCann, H.G., and Scott, D.B. Comparative Fixation of Calcium and Strontium by Synthetic Hydroxyapatite. J. Biol. Chem. 235:2152-6, 1960.

Cohen, E.P., Nylen, M.U., and Scott, D.B. Microstructural Changes Induced in Mammalian Cell Cultures by Omission and Replenishment of a Single Essential Amino Acid. (in press, Exp. Cell Res.)

vonBrand, T., Mercado, T.I., Nylen, M.U., and Scott, D.B. Observations on Function, Composition, and Structure of Cestode Calcareous Corpuscles. Exp. Parasitol. 9:205-14, 1960.

Scott, D.B., Nylen, M.U., and Pugh, M.H. Contact Microradiography in Conjunction with Electron and Optical Microscopy. Norelco Reporter 7:32-35, 1960.

Nylen, M.U., Scott, D.B., and Mosley, V.M. Mineralization of Turkey Leg Tendon. II. Collagen-Mineral Relations Revealed by Electron and X-ray Microscopy. AAAS, Washington, D.C. 1960.

Hampp, E.G., Scott, D.B., and Wyckoff, R.W.G. Morphologic Characteristics of Oral Fusobacteria. J. Bacteriol. 79:716-28, 1960.

Honors and Awards relating to this project:

None

Serial No. NIDR-33
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Microscopic Studies of Special Structures in Dental Enamel.

Principal Investigator: Dr. J. G. Helmcke

Other Investigators: Miss L. Schulz (Guest Worker)

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1 1/2
Professional: 3/4
Other: 3/4

Project Description:

Objectives:

Over the past fifteen years considerable information has been brought out through electron microscopy with regard to the ultrastructural inorganic and organic elements in dental enamel, and their general organization within and between the prismatic units. The time has now been reached when attention should and can intelligently be directed toward configurations such as prism striations, Shreger bands, Retzius' lines and acquired incremental markings which have long been observed at the optical level, but are not understood structurally. One of the long term goals in this laboratory is to investigate these special structures systematically.

Methods Employed:

An integrated light and electron microscopic approach has been used. Areas demonstrating particular structural configurations were characterized exhaustively by all available light optical methods (transmitted, polarized, incident, phase contrast, fluorescent and interference) and then subjected to electron microscopic examination by both standard and stereoscopic methods. Technics for exact localization of specific fields on enamel specimens and cross-referencing systems for microscopic purposes have been used

in these and previous studies but a certain amount of technical refinement is still required. Basic methods of specimen preparation were through fracture or grinding, polishing and etching of surfaces and replication for electron microscopy. As indicated, the underlying approach was to apply in very precise fashion to well-defined areas of specimens all of the microscopic methods available in an attempt to establish the ultrastructural basis for the various optically visible configurations. The present studies have dealt with one structural pattern, the cross striations in the prisms of human enamel.

Major Findings:

Surfaces of non-carious human enamel, prepared either by fracture or by grinding, polishing and etching, were examined by light microscopy, and regions were identified in which prism striation was evident. Series of shadowed positive carbon replicas of these selected areas were made for electron microscopy. The sequential technique provided two types of preparation, for the early films took the form of pseudoreplicas, actually bearing entrapped layers of crystalline and organic material, while the later films were true replicas. The cross striations observed optically in the specimens themselves were recognized in both types of replicas, and a combination of structural features seemed responsible for the configuration. Gentle constrictions were noted along the prisms at the same regular intervals assumed by striations. In addition, certain characteristic arrangements of the ultrastructural components were seen within prisms. In the central prism core the crystals and organic elements were aligned fairly parallel to the prism axis, while in the cortical region they tended to incline outward, angulation being greatest in the widest portions of the prisms. As previously observed, the structures between the prisms resembled those within the prisms, and were oriented obliquely between them. Since quantitative differences in the composition of prisms at regions of cross striation were not evident, it is thought that the variations in structural organization must be responsible for the observed optical effects. If this is true, light microscopy provides an extremely sensitive means of detecting very minute deviations of groups of submicroscopic crystals within a continuous microcrystalline system.

Significance to Dental Research:

The primary significance of this research is to provide further basic information on the structure of mature enamel and to develop further the concepts which deal with the relationships between ultrastructural configurations and their effect on light optical phenomena. The prismatic striations were selected as the starting

point in this study because they are the most commonly observed structures and because the development of clear-cut information on them will have a direct influence on our thinking about the mechanisms involved in the destruction of enamel under attack by caries. If there are no differences in the proportion between organic and inorganic constituents of the enamel prisms as the present studies suggest, and only some variations in their geometrical arrangement, a certain amount of revision in our ideas on caries will be required.

Proposed Course of Project:

Due to lack of personnel, work on this project at this laboratory will be discontinued at this point. However, the studies will be continued at Professor Helmcke's laboratory in Berlin and it is hoped that his collaboration with NIDR will continue.

Part B. included: No

Serial No. NIDR-34
1. Histology & Pathology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Experimental Dental Caries

Principal Investigator: Dr. P. H. Keyes

Other Investigators: None

Cooperating Units: Dr. R. J. Fitzgerald and Dr. H. V. Jordan, Gnotobiotics Section, NIDR.
Dr. Arden Howell, Laboratory of Microbiology, NIDR
Dr. H. R. Hunt, Department of Biology, Michigan State University

Man Years (calendar 1960):
Total: 3 1/4
Professional: 1
Other: 2 1/4

Project Description:

Objectives:

This project has been a continuation of NIDR-7 (1959) in which various factors related to dental caries in hamsters and rats have been studied. Since our previous demonstration that caries is a rather specific streptococcal disease in hamsters, and one that can be transmitted, we have directed our attention to other related microbiological problems, to refinements of methods, and to various types of bioassays. We have:

1. studied the recovery of streptomycin and erythromycin resistant strains of cariogenic streptococci from animals previously inoculated with these strains.

2. induced caries in non-infected animals by the transfer of plaque material from infected carious animals.

3. assessed the caries inducing potential of other acidogenic organisms and other combinations of organisms.

4. demonstrated further the transmission of a cariogenic flora from dam to offspring by means of the "labelled" strains of streptococci and have started a colony of animals which carry these microorganisms.

5. assessed additional agents for their caries inhibitory potential; viz. Thiostrepton, sodium metabisulfite, sodium phytate, oleic acid, in addition to dibasic sodium phosphate, penicillin, and sodium fluoride.

6. studied caries activity in hamsters, passively immunized with rabbit serum, after they had been inoculated with pure cultures of cariogenic streptococci.

In cooperation with Dr. R. J. Fitzgerald (Gnotobiotic Section, NIDR).

1. Both non-infected and naturally infected hamsters have been inoculated with pure cultures of streptococci derived from a carious lesion in a hamster and made resistant to the antibiotics streptomycin and erythromycin. A large number of attempts have been made to recover these organisms from the mouths of animals so inoculated. In one study a selected group of animals was inoculated and recoveries of the inoculated organisms were followed biweekly for 100 days.

2. When non-infected animals have been inoculated with pure cultures, caries has developed more slowly than in animals which have acquired the cariogenic flora from their dams during the suckling period. To overcome this difficulty non-infected animals have been infected with small amounts of plaque material obtained from lesions in caries active animals. In some instances the plaque has been obtained from animals which carry "labelled" cariogenic streptococci. These animals have been tested for the presence of these strains in cooperation with Dr. R. J. Fitzgerald.

3. In collaboration with Dr. R. J. Fitzgerald, hamsters have been inoculated with other acidogenic organisms and combinations of organisms to determine whether caries would develop or be less active. It is possible that some organisms in the mouth may exert an antagonistic influence on the cariogenic flora. Thus animals have been infected with organisms temporarily classified as "atypical" streptococci mixed with other organisms obtained from animals which have not developed active caries.

4. A number of attempts have been made to breed females which carry the labelled cariogenic streptococci in order to follow this flora in the offspring. A small group of animals has been followed with the intent of developing, if possible, a line which will consistently carry these streptococci and which will also be highly caries active.

5. The caries inhibiting potential of several agents besides penicillin and sodium fluoride have been assessed. These include: 1) additional studies with dibasic sodium phosphate (25 gms per kilo of diet); 2) Thiostrepton (Squibb) added to the test diet at a level of 100 and 200 mg per kilo; 3) sodium metabisulfite added to the diet (3 gms per kilo); sodium phytate added to the diet at a level of 15 gms per kilo; oleic acid and hydrogenated coconut oil added to the diet at a level of 1 gm per kilo. The studies with the metabisulfite and oleic acid were collaborative projects with Dr. Harold Jordan.

6. In cooperation with Dr. R. J. Fitzgerald an attempt was made to confer a passive immunity upon hamsters by the injection of the serum from rabbits which had been inoculated with the cariogenic streptococci. There were the following groups: 1) controls which were not infected; 2) animals inoculated with the cariogenic streptococci; 3) animals inoculated with streptococci and injected with normal rabbit serum; 4) animals injected with immune rabbit serum and inoculated with the cariogenic strep.

7. In collaboration with Dr. Arden Howell the mouths of cream and golden hamsters were cultured for the presence of filamentous forms. These organisms seemed to be more prevalent in the mouths of animals which have shown some "resistance" to caries. The possibility that these organisms might be antagonistic to caries producing organisms was considered. These studies were terminated after the illness of Dr. Howell.

Major Findings:

1. When the "labelled" streptococci have been inoculated into animals which carry no apparent cariogenic flora, recovery has been better than when these organisms have been added to an existent flora. We had hoped that the "labelled" streptococci might be introduced into naturally infected animals or combined with pure cultures of other cariogenic streptococci used for inoculations of non-infected animals. They might then

be used as tracers to follow the course of the infection under various experimental programs. However, recovery of the organisms has been so variable that this approach has been discontinued. The difficulty was well illustrated in a series of animals which were cultured regularly over a period of 100 days. All were caged together and similarly inoculated. A positive culture was never obtained from one animal in the group although he developed very rampant caries. In the other animals cultures were initially positive, but fluctuations began to occur so that finally no recovery was possible in most of the animals. However, they all developed caries. The labelled streptococci may have been displaced by the growth of the more virulent strains.

2. When non-infected animals have been inoculated with plaque from carious animals, they have developed active caries. In some the induction and progress of cavitation has compared favorably with that in naturally infected animals. When the plaque has contained "labelled" organisms, recovery of the labelled strains has been good. If this method of inoculation can be somewhat refined and if recovery of the labelled strains can be consistently obtained, this method of inoculation will eliminate the need for breeding infected animals.

3. The other acidogenic organisms tested to date have not induced caries. Combination of organisms, tested with the possibility of demonstrating a caries antagonistic effect, have not shown any such potential under the conditions used.

4. A few females have continued to carry the "labelled strains" in addition to the cariogenic streptococci and have passed this flora to offspring which have developed caries. It may be necessary to breed out a line of animals in which the balance between the "natural" and "labelled" organisms is consistently maintained.

5. Of the additional agents tested for caries inhibitory potential, only sodium metabisulfite has been impressively effective. Thiostrepton, sodium phytate, and oleic acid were not beneficial. Generally poor results were obtained with the dibasic sodium phosphate. These findings are not consistent with other reports on the effect of phosphates. Penicillin and sodium fluoride were used for purposes of comparison and were consistently effective as anticaries agents.

6. No beneficial effect was observed in those hamsters in which passive immunity was attempted. These results were not totally unexpected but were disappointing.

7. No conclusion can be drawn in regard to the significance of the filamentous forms isolated from the mouths of either cream or golden hamsters. Their relationship to caries activity if any remains to be determined.

Significance to Dental Research:

The concept that dental caries is an infection in hamsters continues to be fully supported. Caries does not seem to be a nutritional disease. The need for more information about the alimentary canal flora and that of the mouth is self-evident. It seems safe to predict that significant findings in regard to caries etiology and the basic mechanisms of lesion formation will depend in great measure on our ability to identify the organisms involved and their metabolic processes in relationship to tooth properties. It will be of considerable interest to see if these findings can be extended to humans.

If a satisfactory technic can be developed for the induction of caries by the transfer of plaque, particularly plaque containing labelled organisms, many new approaches to the study of factors related to caries can be undertaken. It will not be necessary to raise infected animals, either those with or without the labelled flora. For the first time it may be possible to follow the effect of inhibitory agents on a known cariogenic flora. Our finding that phosphate has not consistently shown an anticaries effect indicates that more work is needed on this compound. It would seem desirable to know how phosphates affect the flora and what mechanisms might account for the findings in other laboratories.

Proposed Course of the Project:

To study the possibilities of inducing caries by the transfer of plaque. To continue in the collaborative effort with the Section of Gnotobiotics, to define the cariogenic flora and the effect of inhibitors on it. To test the possibilities of preventing caries in animals by a program of active immunization using viable organisms rather than serum.

Part B. included: Yes

Serial No. NIDR-34
1. Histology & Pathology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

Keyes, P.H. The Infectious and Transmissible Nature of Experimental Dental Caries. Findings and Implications. Arch. Oral Biol., 1:304-320, 1960.

Fitzgerald, R.J. and Keyes, P.H. Demonstration of the Etiologic Role of Streptococci in Experimental Caries in the Hamster. J.A.D.A. 61:9-19, 1960.

Honors and Awards relating to this project:

None

Serial No. DTDR-15
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Electron Microscopic Studies of Dental Calculus.

Principal Investigator: Dr. Jørgen Theilade

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1/4
Professional: 1/4
Other: 0

Project Description:

Objectives:

This laboratory has been interested in calcification as it occurs in normal tissues and in various pathological conditions. Dental calculus is an ectopic, and in a sense, pathological calcification which readily lends itself to investigation. This project is a continuation of previous investigations performed in this laboratory on mature and developing human dental calculus and on dental calculus from germfree rats. The basic objectives are further investigation of the organic and inorganic structure of mature calculus, and the mechanisms involved in deposition and mineralization.

Methods Employed:

(1) Samples of dental calculus have been removed from human patients and are being processed for optical, electron microscopic and microradiographic examination.

(2) Early deposits have been collected on plastic strips which are worn on the lingual surfaces of lower anterior teeth in human subjects. The strips bearing deposits have been removed after intervals varying from 2 hours to 2 days and

for electron microscopy. Similar specimens will be prepared for comparative optical microscopy.

(3) Dental calculus has been removed from conventionally bred and germ-free rats and prepared for examination in the electron microscope.

Major Findings:

Thus far, preliminary results are available only from the developing human calculus. One-and 2-day deposits on the plastic strips show an initial collection of epithelial cells with bacteria present in extremely low numbers. At 9 days the number of bacteria has increased and they seem to be concentrated around the degenerating epithelial cells. Beginning calcification has been observed in the intermicrobial substance but not in the degenerating epithelial cells.

Significance to Dental Research:

Apart from the basic knowledge which may be obtained as to the calcification process in general, information on the attachment and formation of dental calculus is of importance for our understanding of etiological factors in periodontal disease.

Proposed Course of Project:

This project was activated in the fourth quarter of the calendar year, and will continue along the lines described.

Part B. included: No

Serial No. NIDR-36
1. Histology & Pathology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Histochemical Studies of Connective Tissue.

Principal Investigator: Dr. Harold M. Fullmer

Other Investigators: None

Cooperating Units: Dr. G. R. Martin, Laboratory of Biochemistry, NIDR
Dr. J. J. Burns, Laboratory of Clinical Pharmacology, NHI
Dr. L. T. Kurland, Epidemiology Branch, NINDB
Dr. H. Seidler, Epidemiology Branch, NINDB
Dr. R. S. Krooth, Laboratory of Cell Biology, NIAID
Dr. F. W. Wertheimer, Clinical Investigations Branch, NIDR

Man Years (calendar year 1960)
Total: 3
Professional: 1
Other: 2

Project Description:

Objectives:

To determine the chemical composition and the reactive groups on normal and diseased connective tissues through the use of histochemical methods.

Methods Employed:

Human and animal tissues are obtained from autopsy and surgical material. Specimens to be sectioned are embedded after either freeze-drying or chemical fixation and dehydration. Under controlled conditions, the sections are exposed to various reagents designed to determine the site and quantity of a reactive group or substance.

Major Findings:

1. In 1958 a new stain, the peracetic acid-aldehyde fuchsin-Halmi, was developed which led to the discovery of a new connective tissue fiber. This has been named the oxytalan fiber and is located principally in areas of stress such as the periodontal membranes, ligaments and tendons. The fibers are found in several animals as well as in man. They have protein and mucopolysaccharide components. The studies of histogenesis of these fibers have been continued. Their development appears to be intimately associated with mucopolysaccharide situated between bundles of collagen, and their histochemical reactions suggest a relation to elastic fibers.

2. The development of another new stain, the peracetic acid-orcein-Halmi, has permitted the demonstration of oxytalan fibers in mucous connective tissues such as developing tooth pulps and umbilical cords. Use of this technique has eliminated interference with definition of the fibers ordinarily produced by the reactions of other types of stains with the surrounding mucopolysaccharide.

3. The histogenesis of oxytalan fibers in dental granulomas, radicular cysts and in the supportive stroma of ameloblastomas was studied. They were observed to develop in these pathological tissues as they do during embryogenesis. This is interesting because it demonstrates an inherent capacity of connective tissue cells of the periodontium to produce oxytalan fibers (a) in an adult, and (b) under pathological conditions.

4. A considerable amount of time was expended in efforts to define the position of oxytalan in relation to other connective tissue fibers. This was achieved by the study of (a) connective tissue fiber types in periodontal membranes of several animals (b) histogenesis of elastic fibers in human embryos, and (c) oxytalan and elastic fibers in scleroderma.

The following data foster the hypothesis that oxytalan fibers are an elastic-like tissue possibly representing an immature or a specially modified elastic fiber. (A) The presence of typical elastic fibers in periodontal membranes of the dog, sheep, swine and the steer was confirmed. These were in an array analogous to oxytalan fibers in man. (B) Typical oxytalan fibers were found especially about the apical and middle thirds of periodontal membranes of these animals in an array analogous to that of elastic fibers in the superior thirds of periodontal membranes of these animals and to that of oxytalan fibers in man. Also, oxytalan fibers frequently appeared to interchange for elastic fibers in

the superior thirds of some of these membranes. (C) Developing elastic fibers are demonstrable with the peracetic acid-aldehyde fuchsin-Halmi and the peracetic acid-orcein-Halmi stains before they can be visualized with regular elastic tissue stains. (D) Very fine extensions of elastic fibers such as those at the dermal-epidermal junction are demonstrable with the peracetic acid-aldehyde fuchsin-Halmi stain and not with regular elastic tissue stains. (E) Oxytalan fibers are resistant to elastase; a previous report that developing elastic fibers are also resistant to elastase was confirmed. Peracetic acid oxidation of either oxytalan fibers or pre-elastic fibers renders them equally susceptible to elastase digestion. (F) Peracetic acid oxidation of either pre-elastic or oxytalan fibers renders them equally susceptible to the digestion of the aldehyde fuchsin-stainable component of the fibers by β-glucuronidase. (G) Rarely do both fine elastic fibers take the same locations as oxytalan fibers in the periodontal membrane of man. (H) True elastic fibers were found in some areas of collagen sclerosis in the periodontal membrane of a patient who died of scleroderma. It is plausible to present that oxytalan fibers undergo developmental alterations and possible retain relatively as does collagen in these lesions. (I) Oxytalan fibers are demonstrated by three of five elastic tissue stains provided they are oxidized with peracetic acid.

5. Our data indicated that the eosinophilic component of oxytalan fibers was a mucopolysaccharide. Since the staining reaction was induced by a peracetic acid oxidation, it was decided to investigate the effects of a peracetic acid oxidation on mucopolysaccharides. It was found for the first time that:

   (a) β-glucuronidase digests certain mucopolysaccharides if they are pre-oxidized with peracetic acid. The periodic acid-Schiff stain is rendered non-reactive in these preparations.

   (b) Peracetic acid oxidation of tissue sections accelerates digestion of some mucopolysaccharides by trypsin and pepsin.

   (c) The peracetic acid-aldehyde fuchsin-Halmi stain demonstrates a mucopolysaccharide(s) heretofore not observed histochemically. Its chemical identity is unknown.

   (d) Umbilical cord and developing tooth pulps contain a mucopolysaccharide reactive with the peracetic acid-aldehyde fuchsin-Halmi stain that does not induce metachromasia with basic dyes.

(e) The peracetic acid-aldehyde fuchsin-Halmi and the Hale stains demonstrate a mucopolysaccharide(s) in mast cells that does not react metachromatically with basic dyes.

The mechanisms whereby peracetic acid induces staining of mucopolysaccharide(s) by aldehyde fuchsin, and alter mucopolysaccharides so that they are susceptible to various mucopolysaccharases and some proteolytic enzymes is unknown.

6. Collaboration with the NIH and Dr. George Martin has continued in our investigations of connective tissues in regard to the mechanism of action of vitamin C. We have attacked this problem by the administration of various compounds to scorbutic guinea pigs. Thereafter the weight gain, survival rate, extent of hemorrhage, and characteristics of dentinogenesis were studied. Contrary to previous reports of others, D-ascorbic acid was found to have some vitamin C action. It was found to permit dentinogenesis, weight gain and survival of the animals, but it failed to prevent the typical hemorrhages. Recent additional work disclosed that 3-methylcholanthrene permitted dentinogenesis, prevented hemorrhages, and restored mucopolysaccharides in the gingivae, but it did not foster weight gain. It is considered particularly significant that 3-methylcholanthrene is not structurally related to vitamin C. These data permit the nurture of the notion that vitamin C has multiple actions and that certain compounds may replace some but not all of the actions of vitamin C.

7. While using a section of abdominal skin from a patient who died of amyotrophic lateral sclerosis as a control, it was discovered that it reacted abnormally in response to the peracetic acid-aldehyde fuchsin-Halmi stain. The cooperation of investigators in the NINDB was sought and subsequently dermal biopsies from 46 patients with amyotrophic lateral sclerosis have been studied. As a result, it was demonstrated for the first time that a characteristic connective tissue disorder exists in approximately 60% of patients with ALS. The disorder is characterized by (a) a distinctive elastosis, (b) usually by an increase of mucopolysaccharide, (c) a degeneration and elastosis of arrector pili muscles, (d) focal areas of apparently regenerated connective tissues, and (e) several characteristic changes in collagen. These changes were found in a number of patients from the United States and from Guam. Individuals severely afflicted with ALS manifest the disorder more frequently than those less severely afflicted. Since the skin specimens from several patients having various neurological disorders resulting in loss of motor innervation to the biopsied site did not manifest the changes ascribed to ALS, a primary

disorder of connective tissue in this disease is suggested rather than being secondary to loss of motor innervation. The characteristic changes of connective tissues found in ALS are such that it is doubtful that they would have been observed without the use of the peracetic acid-aldehyde fuchsin-Halmi stain.

8. The epithelial attachment (secondary dental) cuticle is located on the surface of the epithelial attachment and apparently connects the epithelium to the tooth. During the course of experiments on periodontal membranes the cuticle was observed to be selectively stained with orcein. In collaborative study with Dr. F. W. Wertheimer, it was found that the cuticle contains a peculiar type of carbohydrate which is demonstrated with the Bauer and Casella stains, but not with the periodic acid-Schiff method. It was further found to contain arginine and tryptophane, and probably histodine and lysine, and to be unreactive to stains for tyrosine. It is possible that this unusual carbohydrate-protein complex is required for a special function of binding tooth to gingiva.

Significance to Dental Research:

Periodontitis and periodontosis are diseases of connective tissues. Information gained about the normal connective tissues and their alterations in the diseased states will contribute to a better basic understanding of the pathological processes involved in periodontal disease. The ultimate importance of the discovery of a new connective tissue fiber in periodontal tissues and in other tissues subjected to stress is still unknown. It is certain to broaden our view of elastic-like tissues, because now that pre-elastic tissue cannot be demonstrated (stained), these fibers may now be sought in lesions of arteriosclerosis, various connective tissue disorders and in connective tissue neoplasms.

Since vitamin C is required for the production of collagen and dentin, it is useful to know the mechanism whereby this requirement is met. As more information is obtained about forces that promote the formation and degraduation of connective tissues, it is plausible to presume that we may be better able to maintain connective tissues in a state of health, and to treat them if they are diseased. Since a considerable percentage of patients with amyotrophic lateral sclerosis have a connective tissue disorder that on the basis of preliminary studies appears to be a primary part of the disease, a new concept of the nature of this disease must be developed.

Part A. (continued) Serial No. NIDR-36

Proposed Course of Project:

Continued attention will be directed to the histochemical study of normal and diseased connective tissues and their alterations during development and with age.

Part B. included: Yes

Serial No. NIDR-36
1. Histology & Pathology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

Fullmer, H.M. A Comparative Histochemical Study of Elastic, Pre-elastic and Oxytalan Connective Tissue Fibers. J. Histochem. & Cytochem. 8:290-295, 1960.

Fullmer, H.M. Effect of Peracetic Acid on the Enzymatic Digestion of Various Mucopolysaccharides: Reversal of the Periodic Acid-Schiff Staining Reaction of Mucin. J. Histochem. & Cytochem. 8:113-121, 1960.

Fullmer, H.M. Observations on the Development of Oxytalan Fibers in Dental Granulomas and Radicular Cysts. A.M.A. Arch. Path. 70:59-67, 1960.

Fullmer, H.M., Seidler, H.D., Krooth, R.S., and Kurland, L.T. A Cutaneous Disorder of Connective Tissue in Amyotrophic Lateral Sclerosis: A Histochemical Study. Neurology 10:717-724, 1960.

Fullmer, H.M. and Wertheimer, F.W. Orcein as a Selective Stain for Epithelial Attachment Cuticle. Stain Technol. 35:156-159, 1960.

Wertheimer, F.W. and Fullmer, H.M. The Epithelial Attachment (secondary dental) Cuticle: Bauer and Casella Positive, Periodic Acid-Schiff Negative. J. Histochem. & Cytochem. 8:442-443, 1960.

Fullmer, H.M., Martin, G.R., and Burns, J.J. Role of Ascorbic Acid in the Formation and Maintenance of Dental Structures. Ann. New York Acad. Sci. (in press) 1961.

Martin, G.R., Fullmer, H.M., and Burns, J.J. Observation on the Effect of 3-methylcholanthrene in Scorbutic Guinea Pigs. Proc. Soc. Exper. Biol. & Med. (in press) 1961.

Fullmer, H.M. A Histochemical Study of Periodontal Disease in the Maxillary Alveolar Processes of 135 Autopsies. J. Periodontol. (in press) 1961.

Honors and Awards relating to this project:

None

Serial No. NIDR-37
1. Histology & Pathology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Development and Application of Histochemical Methods in the Study of Hard and Soft Tissues.

Principal Investigator: Dr. M. S. Burstone

Other Investigators: None

Cooperating Units: Dr. E. Weisburger (NCI), Dr. S. Wollman (NCI) and Dr. S. Spicer (NIAMD)

Man Years (calendar year 1960)
Total: 2 1/4
Professional: 1
Other: 1 1/4

Project Description:

Objectives:

The objectives of this project are the development and modification of histochemical methods and their application in histopathological and histological studies.

Methods Employed:

The methods employed are microscopic histochemical procedures. These techniques enable the visualization, for example, of sites of enzyme activity in normal and abnormal bone, and in various types of neoplasms. Among the enzyme procedures being used are those which demonstrate esterase, phosphatase, sulfatase, glucuronidase, peptidase, dehydrogenase, and cytochrome oxidase. New substrates for the demonstration of aminopeptidase have been developed in conjunction with Dr. E. Weisburger (NCI).

Technique for the visualization of amino acids and proteins are also used as general survey methods. These procedures employ dinitrofluorobenzene, ninhydrin, alloxan, and dimethylaminobenzaldehyde.

Part A. (Continued) Serial No. NIDR-37

Major Findings:

1. A new finding, namely the presence of high cytochrome oxidase activity in osteoclasts has been made. This in conjunction with previously made observations further tends to confirm that these cells exhibit high metabolic activity.

2. Several new aminopeptidase substrates have been developed. Among these are derivatives of nitronaphthylamine and aminochrysene. These compounds may offer suggestions for improved substrate design.

3. A number of new diazonium salts have been prepared from nitronaphthylamines, azo dyes, and carbazole derivatives. These are of value in certain specific azo dye reactions such as the post coupling technique.

4. Modification of the cytochrome oxidase technique has proved of value in identifying certain specific cell types such as gastric "chief" cells and neurons of the central and peripheral nervous systems.

Significance to Dental Research:

Microscopic histochemical techniques reveal biochemical sites of enzyme and other activities in the oral tissues. Such sites are not discernable with routine stains. The histochemical approach represents a more accurate chemical reflection of pathological processes as contrasted with interpretations based upon non-specific staining reactions.

Proposed Course of Project:

Discontinued due to transfer of principal investigator.

Part B. included: Yes

Serial No. NIDR-37
1. Histology & Pathology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

Burstone, M.S. Histochemical Methods for Protein Detection. Chapter in Handbuch der Histochemie. Band III, pp. 244-284. G. Fischer Verlag, Stuttgart, 1959.

Burstone, M.S. Histochemical Localization of Oxidase Activity in the Mitochondria of the Human Heart. Nature 184:476-477, Aug. 1959.

Hunter, R.L. and Burstone, M.S. The Zymogram as a Tool for Characterization of Substrate Specificity. J. Histochem. & Cytochem. 8:58-62, Jan. 1960.

Burstone, M.S. Histochemical Demonstration of Cytochrome Oxidase with New Amine Reagents. J. Histochem. & Cytochem. 8:63-70, Jan. 1960.

Burstone, M.S. Histochemical Observations on Enzymatic Processes in Bones and Teeth. Chapter in the Metabolism of Oral Tissues. N.Y. Acad. Sci. 85:431-444, March 1960.

Burstone, M.S. Histochemical Demonstration of Succinic Dehydrogenase Activity in Osteoclasts. Nature 185:866, March 1960.

Burstone, M.S. Postcoupling, Noncoupling, and Fluorescence Techniques for the Demonstration of Alkaline Phosphatase. J. Nat. Cancer Inst. 24:1199-1217, May 1960.

Burstone, M.S. Histochemical Demonstration of Cytochrome Oxidase Activity in Osteoclasts. J. Histochem. & Cytochem. 8:225-226, May 1960.

Burstone, M.S. Hydrolytic Enzymes in Dentinogenesis and Osteogenesis. Chapter in Calcification in Biological Systems. Am. Assoc. Adv. Sci. Monograph. 217-243, 1960.

Honors and Awards relating to this project:

None

NATIONAL INSTITUTE OF DENTAL RESEARCH

Individual Project Reports
Calendar Year 1960

EPIDEMIOLOGY & BIOMETRY BRANCH:

38. A. L. Russell and C. L. White: The Epidemiology of Dental Caries - Including the Fluoride-Dental Caries Relationship

39. A. L. Russell and C. L. White: Epidemiology of Oral Diseases among Alaskan Natives

40. N. W. Littleton: Epidemiology of Oral Diseases among Ethiopians

41. E. C. Leatherwood and J. C. Greene: Epidemiology of Oral Diseases in Ecuador

42. A. L. Russell and E. C. Leatherwood: Epidemiology of Oral Diseases in Vietnam

43. C. J. Donnelly: Epidemiology of Oral Diseases in Colombia, South America

44. E. C. Leatherwood: Epidemiology of Oral Diseases in Thailand

45. C. J. Donnelly and N. W. Littleton: Epidemiology of Fractured Incisors

46. A. L. Russell and C. L. White: Relation Between Dental Caries and Periodontal Diseases

47. C. J. Donnelly: Study of Periodontal Disease and Caries in Adventists Families

Serial No. NIDR-38
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: The Epidemiology of Dental Caries - Including the Fluoride-Dental Caries Relationship

Principal Investigator: Dr. A. L. Russell

Other Investigator: Mr. C. L. White

Cooperating Units: Board of Education, City of Grand Rapids; City-County Health Department, Grand Rapids, Michigan; Michigan Department of Health; Montgomery and Prince Georges Counties, Maryland

Man Years (calendar year 1960):

Total:	2 1/4
Professional:	1/2
Other:	1 3/4

Project Description:

Objectives:

1. To observe the effects of fluoridation of a community water supply on children whose initial exposure to fluoride occurred at different age levels.

2. To define the epidemiological characteristics of dental caries.

3. To obtain basic information concerning the nature of dental caries and the dental tissues susceptible to attack so that present methods of control may be perfected and, possibly, new methods may be evolved.

Methods Employed:

Direct examination of population groups with subsequent analysis of findings to determine relative risks under varying conditions.

Part A (continued) Serial No. NIDR-38

Major Findings:

1. Progressive reduction in the prevalence of dental caries in the Grand Rapids population under study continued during the past year. Sodium fluosilicate, a less expensive compound, appears as effective as sodium fluoride when used in fluoridation of public water supplies.

2. After seven years of fluoridation in Montgomery and Prince Georges Counties, Maryland, there has been a significant reduction in dental caries. From children up through age nine whose teeth largely developed during the use of fluoridated water, the curve for DMF means closely approximates an expectancy curve obtained from an area whose population uses a water containing natural fluorides. Two significant aspects of the fluoride-dental caries hypotheses appear to be:

   a. Time available for the accumulation of fluoride in a tooth prior to eruption.

   b. Time at risk, or the length of time a tooth is exposed to attack by dental caries.

Significance to Dental Research:

Findings from this study are expected to expand present knowledge of the beneficial effects of controlled fluoride ingestion on the dentition. Through detailed analysis, an attempt will be made to secure information on the action of fluoride during the various formative periods of the teeth. Inferences can be made on the duration of the inhibitory effects of fluoride on dental caries.

Proposed Course of Project:

Studies on the epidemiology of dental caries will continue. Concentrated effort will be made in areas where basic information concerning the action of fluoride in humans is equivocal or entirely lacking. Examination of these population samples will continue until:

1. The full inhibitory effect of fluoride on dental caries becomes evident in each age group and inferences can be made as to the duration of this effect.

2. The prevalence of fluorosis has reached a maximum.

3. Information is available on the modes of action of fluoride on the dental tissues.

Part B included: No

Serial No. NIDR-39
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Epidemiology of Oral Diseases among Alaskan Natives

Principal Investigator: Dr. A. L. Russell

Other Investigator: Mr. C. L. White

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense

Man Years (calendar year 1960):
Total: 3/4
Professional: 1/2
Other: 1/4

Project Description:

Objectives:

To determine the prevalence of dental caries, periodontal diseases, and malocclusion among Alaskan natives and to investigate possible relationships of these disorders to certain nutritional and geographic characteristics of this group.

Methods Employed:

Direct examination of members of the Alaskan National Guard. Subsequently, statistical analysis of data obtained to determine relative risks under varying conditions.

Major Findings:

Dental examinations of approximately 700 Eskimo men of the Alaskan National Guard revealed significant information concerning oral health in this population group. Preliminary analysis of data indicated interesting differences when findings from "primitive" groups were compared with those from groups living under more civilized conditions. Dental caries was virtually non-existant in individuals from remote villages. Many of the primitive groups were essentially free of periodontal diseases. Other similarly primitive groups exhibited a uniformly prevalent, severe gingivitis.

Part A (Continued) Serial No. NIDR-39

Contrary to generally accepted theory, this gingivitis demonstrated little tendency toward progression into chronic destructive periodontal diseases even in the absence of personal oral hygiene or professional dental care. In contrast to these findings in the more "primitive" groups, Eskimos that had lived for some time under relatively "civilized" conditions, demonstrated prevalence and severity of oral diseases quite comparable to those seen in average male population groups within the United States.

Significance to Dental Research:

The spectrum of living conditions within this Eskimo group affords a unique opportunity to study the influence of "civilization" on oral diseases. Findings from the "primitive" groups are relatively uninfluenced by professional dental care. An opportunity is also present for evaluating oral diseases in terms of the various nutritional patterns included within this population sample.

Proposed Course of Study:

Data from this study undoubtedly represents a basis from which suggestions may be formulated for improving the oral health status of the Alaskan Peoples. Furthermore, analysis of these data should contribute to our knowledge of the epidemiological characteristics of oral diseases. It is planned to extend this project to include a study of children from "primitive" village schools.

Part B included: Yes

Serial No. NIDR-39

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Russell, Albert L., Littleton, Norman W., Leatherwood, Ernest C., Sydow, Gunnar E., and Greene, John C. Dental Surveys in Relation to Nutrition. Public Health Reports. 75: 717-23, August 1960.

Honors and Awards relating to this project:

None

Serial No. NIDR-40
1. Epidemiology & Biometry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Epidemiology of Oral Diseases among Ethiopians

Principal Investigator: Dr. Norman W. Littleton

Other Investigators: None

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense

Man Years (calendar year 1960):
Total: 1
Professional: 3/4
Other: 1/4

Project Description:

Objectives:

1. To determine the oral health status in a representative sample of Ethiopians; in particular, to determine the prevalence and epidemiological characteristics of dental caries, periodontal diseases, fluorosis, malocclusion and attrition.

2. To relate these findings to medical, nutritional, and environmental factors in an effort to establish possible associations.

Methods Employed:

Direct examination of an Ethiopian sample with subsequent analysis of examination data to determine relative risk under varying conditions.

Major Findings:

The prevalence of dental caries in the Ethiopian sample was very low. This low prevalence occurred in a population apparently subsisting on a diet traditionally high in natural carbohydrates but low in sugar. Information on fluoride levels tended to indicate that the relative freedom from dental caries was not entirely dependent on fluoride ingestion. Periodontal diseases were highly prevalent and severe in this population. Nutritional

Part A (Continued) Serial No. NIDR-40

factors appeared to exert only a minor influence on the status of the periodontal tissues. Positive associations existed between the severity of periodontal diseases and the state of oral cleanliness in this group.

Significance to Dental Research:

This project presented an opportunity to study a population in which little was known concerning oral health status, and one in which the effects of personal oral hygiene and professional dental care would be minimal. Findings have been evaluated in terms of certain environmental, medical, biochemical, and nutritional data in an effort to establish possible associations.

Proposed Course of Project:

1. To complete the tabulation and analysis of the data.

2. To utilize findings to formulate possible suggestions for improving oral health conditions within Ethiopia.

3. Through correlating these findings with those of other surveys, attempt to expand information concerning the epidemiological characteristics of oral diseases - particularly dental caries and periodontal diseases.

Part B included: Yes

Serial No. NIDR-40

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Russell, Albert L., Littleton, Norman W., Leatherwood, Ernest C., Sydow, Gunnar E., and Greene, John C. Dental Surveys in Relation to Nutrition. Public Health Reports. 75:717-23, August, 1960.

Honors and Awards relating to this project:

None

Serial No. NIDR-41
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Epidemiology of Oral Diseases in Ecuador

Principal Investigators: Dr. Ernest C. Leatherwood and
Dr. John C. Greene

Other Investigators: None

Cooperating Units: Office of the Chief Dental Officer, USPHS
Interdepartmental Committee on Nutrition for National Defense

Man Years (calendar year 1960):

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases in a sample population from Ecuador, and to relate these findings to certain environmental, medical, biochemical, and nutritional data in an attempt to establish possible associations.

Methods Employed:

Direct examination of population groups followed by statistical analysis of findings to determine relative risks under varying conditions.

Major Findings:

Analysis of these data are still in progress. Preliminary tabulation of findings indicates that dental caries prevalence in the Ecuadorian sample varied in different regions of the country. This appeared to be associated with differences in fluoride intake. Dental caries prevalence also appeared to be somewhat related to the reported frequency of sugar consumption. The prevalence and severity of periodontal diseases were quite high.

Part A (Continued) Serial No. NIDR-41

Significance to Dental Research:

This project presents an opportunity to expand information on the epidemiological characteristics of oral diseases. Further, possible associations may be established between the occurrence of oral disease and environmental, medical, biochemical, and nutritional factors.

Proposed Course of Project:

Tabulation and analysis of data obtained, and correlation of findings with those from other surveys in an attempt to improve present knowledge concerning the occurrence of oral diseases in various population groups.

Part B included: Yes

Serial No. NIDR-41

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Russell, Albert L., Littleton, Norman W., Leatherwood, Ernest C., Sydow, Gunnar E., and Greene, John C. Dental Surveys in Relation to Nutrition. Public Health Reports. 75:717-23, August, 1960.

Honors and Awards relating to this project:

None

Serial No. NIDR-42
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Epidemiology of Oral Diseases in Vietnam

Principal Investigators: Dr. A. L. Russell and
Dr. Ernest C. Leatherwood

Other Investigators: None

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense

Man Years (calendar year 1960):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases in a sample population from Vietnam and to relate these findings to certain environmental, medical, biochemical, and nutritional data in an attempt to establish possible associations.

Methods Employed:

Direct examination of population groups followed by statistical analysis of findings to determine relative risk under varying conditions.

Major Findings:

Analysis of these data are still in progress. However, preliminary tabulation indicates that prevalence of dental caries was generally low. While the prevalence of periodontal diseases was widespread in the general population, these conditions were extremely severe in individuals living in certain mountainous areas. These findings occurred in a population subsisting on a soft, rice diet with indications of marginal intakes of thiamine, riboflavin, and Vitamin A.

Part A (Continued) Serial No. NIDR-42

Significance to Dental Research:

This project presents an opportunity to expand present information on the epidemiological characteristics of oral diseases. Furthermore, possible associations may be established between the occurrence of oral diseases and environmental, medical, biochemical, and nutritional factors.

Proposed Course of Project:

Tabulation and analysis of the data obtained, and correlation of findings with those from other surveys in an attempt to improve present knowledge concerning the occurrence of oral diseases in various population groups.

Part B included: Yes

Serial No. NIDR-42

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Russell, Albert L., Littleton, Norman W., Leatherwood, Ernest C., Sydow, Gunnar E., and Greene, John C. Dental Surveys in Relation to Nutrition. Public Health Reports. 75:717-23, August, 1960.

Honors and Awards relating to this project:

None

Serial No. NIDR-43
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Epidemiology of Oral Diseases in Colombia, South America

Principal Investigator: Dr. Charles J. Donnelly

Other Investigators: None

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense

Man Years (calendar year 1960):
Total: 1
Professional: 1/4
Other: 3/4

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases in a sample population from Colombia and to relate these findings to certain environmental, medical, biochemical, and nutritional data in an attempt to establish possible associations.

Methods Employed:

Direct examination of population groups followed by statistical analysis of findings to determine relative risk under varying conditions.

Major Findings:

Tabulation and analysis of data has not progressed sufficiently to allow statements on findings.

Significance to Dental Research:

This project presents an opportunity to expand present information on the epidemiological characteristics of oral diseases. Furthermore, possible associations may be established between the occurrence of oral diseases and environmental, medical, biochemical, and nutritional factors.

Part A (Continued) Serial No. NIDR-43

Proposed Course of Project:

Tabulation and analysis of the data obtained, and correlation of findings with those from other surveys in an attempt to improve present knowledge concerning the occurrence of oral diseases in various population groups.

Part B included: No

Serial No. NIDR-44
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Epidemiology of Oral Diseases in Thailand

Principal Investigator: Dr. Ernest C. Leatherwood

Other Investigators: None

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense and Walter Reed Army Medical Center, Institute of Dental Research

Man Years (calendar year 1960):
Total: 1/4
Professional: 1/4
Other: 0

Project Description:

Objectives:

1. To determine the oral health status in military and civilian samples in Thailand; in particular, to determine the prevalence and epidemiological characteristics of dental caries, periodontal diseases, fluorosis, malocclusion, and attrition.

2. To relate these findings to medical, nutritional, and environmental factors in an effort to establish possible associations.

Methods Employed:

Direct examination of military and civilian samples in Thailand with subsequent analysis of data to determine relative risk under varying conditions.

Major Findings:

Examinations are in progress.

Significance to Dental Research:

This project presented an opportunity to evaluate the prevalence of oral diseases in the population under study. It also offered

Part A (Continued) Serial No. NIDR-44

the opportunity to evaluate the oral health status in terms of certain biochemical, nutritional, and environmental data in an effort to establish possible associations.

Proposed Course of Project:

1. Upon completion of examinations, to tabulate and analyze the data.

2. Attempt to establish associations within these data between dental findings and the various nutritional, physical, and environmental characteristics of the populations under study.

3. Attempt to correlate these findings with those of other similar surveys in an effort to expand information concerning the epidemiological characteristics of oral diseases.

Part B included: No

Serial No. NIDR-45
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Epidemiology of Fractured Incisors

Principal Investigator: Dr. C. J. Donnelly

Other Investigator: Dr. N. W. Littleton

Cooperating Units: None

Man Years (calendar year 1960):
Total: 3/4
Professional: 1/2
Other: 1/4

Project Description:

Objectives:

To determine, by tooth type, the prevalence of fractured permanent incisors and the extent of damage to the dental tissues resulting from accidents.

Methods Employed:

The prevalence of fractured incisors and the extent of damage is being ascertained by direct observation of several school populations including all grades - kindergarten through high school. By interview, information is being obtained concerning the scene, time and circumstances of the accident. The age, sex, predominant hand, presence of restoration, and student's activity at the time of the accident also is being recorded.

Major Findings:

In the populations thus far surveyed, there are many more upper incisors involved than lower. The most susceptible tooth in these populations is the upper left central incisor. More fractures are being recorded than reported in some of the earlier literature. Many of the cases in which the fracture extends into the dentin were not restored at the time of examination.

Part A (Continued) Serial No. NIDR-45

Significance to Dental Research:

Fractured anterior teeth are disturbing to the child and to the parent and often are difficult to restore satisfactorily. It is hoped that the study will focus attention on this problem by defining its proportions, and that a knowledge of the circumstances of the accidents will suggest precautionary measures.

Proposed Course of Project:

Data will be analyzed to determine prevalence and causative factors.

Part B included: No

Serial No. NIDR-46

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Relation Between Dental Caries and Periodontal Diseases

Principal Investigator: Dr. A. L. Russell

Other Investigator: Mr. Carl L. White

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

To study possible correlations between dental caries, fluoride, and periodontal diseases.

Methods Employed:

Analysis of data obtained from studies on caries and periodontal diseases conducted by the Epidemiology and Biometry Branch.

Major Findings:

1. Preliminary findings indicate that there is no significant difference in the occurrence of periodontal diseases about teeth with or without dental caries.

2. Periodontal tissues are not harmed by use of a fluoride-bearing domestic water.

Significance to Dental Research:

This project expands the program of the Epidemiology and Biometry Branch through statistical investigation of data available from other projects.

Part A (Continued) Serial No. NIDR-46

Proposed Course of Project:

To continue to expand the study as outlined.

Part B included: Yes

Serial No. NIDR-46

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Accepted for publication by New York State Dental Journal

Russell, A. L., and White, Carl L. Some Relations Between Dental Caries Experience And Active Periodontal Disease In Two Thousand Adults

Honors and Awards relating to this project:

None

Serial No. NIDR-47
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Study of Periodontal Disease and Caries in Adventists Families

Principal Investigator: Dr. C. J. Donnelly

Other Investigators: None

Cooperating Units: Conferences of Seventh Day Adventists in Indiana, North Dakota, Wisconsin, Michigan, Oklahoma, Southern New England, Chesapeake, and Georgia-Cumberland

Man Years (calendar year 1960):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

1. To investigate the influence of familial factors and of geographic location on periodontal diseases and dental caries among Adventists families.

2. To determine if the dental caries rate among Adventists children is lower than among comparable non-Adventists children.

3. To study the prevalence and severity of periodontal disease in children and adults from a related group of individuals.

Methods Employed:

Direct examination of parents and children attending Seventh Day Adventists Camp Conferences in different parts of the United States. The data will be treated statistically to determine the influence of familial factors, geographic location and Adventists health reforms.

Part A (Continued) Serial No. NIDR-47

Major Findings:

Children examined at Adventists camps show a consistently low caries rate regardless of the part of the country in which they live. A controlled study of Adventist and public school children with seven years of fluoride experience suggests that the caries inhibition noted in Adventists children is similar to and independent of fluoride ingestion but is not greater than inhibition associated with the ingestion of fluoride. No geographic differences were apparent in the periodontal status of the Adventists examined at the seven camps. There is a tendency for the offspring to reflect the periodontal status of their parents.

Significance to Dental Research:

1. The low-caries rate consistently found among Adventists children may be related to the dietary recommendations of the "health reform" suggested by the Adventists Church. Further study might add to our information on the influence of diet on caries rates.

2. By examining Adventists in different parts of the country, some information will be obtained on the influence of geographic location on caries and periodontal disease. By determining if one parent exerts more influence than the other on the periodontal status of the offspring, additional information may be gained about the factors influencing the severity of periodontal disease.

Proposed Course of Study:

1. The controlled study of caries prevalence among Adventist and public school children will be extended to a nonfluoride area to eliminate the influence of fluoride on teeth.

2. Data collected at the Adventist camps will be analyzed for any possible influence of familial or geographic factors.

Part B included: Yes

Serial No. NIDR-47

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Accepted for publication by Public Health Reports:

Donnelly, Charles J. Dental Caries Experience in Adventists and Non-Adventists Children in Five Counties in Maryland

Honors and Awards relating to this project:

None

NATIONAL INSTITUTE OF DENTAL RESEARCH

Individual Project Reports
Calendar Year 1960

CLINICAL INVESTIGATIONS:

48. C. J. Witkop, Jr.: Tri-racial Isolates in Eastern United States.

49. C. J. Witkop, Jr.: Hereditary Defects of Enamel and Dentin (Michigan Study).

50. C. J. Witkop, Jr.: Genetic Study in Southern Maryland.

51. C. J. Witkop, Jr.: Sickle Cell Anemias, Other Inherited Hematological Disorders, and Blood Groups.

52. J. D. Niswander: Oral Aspects of the Results of Consanguineous Marriage in Hiroshima and Nagasaki, Japan.

53. R. M. Stephan: Studies of the Oral Microbiota and Oral Environment in Relation to Dental and Systemic Diseases.

54. R. M. Stephan: Interrelationships Between Local and Systemic Factors in the Etiology of Dental Disease in Experimental Animals.

55. E. J. Driscoll: General Anesthesia (Dental) Baseline Data.

56. P. J. Coccaro: Serial Cephalometric Study of Postural Changes in Maxillary and Mandibular Full Dentures.

57. P. J. Coccaro: Serial Cephalometric Analysis of Facial Height with Natural Dentition and Following Full Denture Construction.

58. P. N. Baer: Studies on Experimental Calculus Formation.

59. P. N. Baer: Studies on Periodontal Disease in the Mouse.

60. H. R. Stanley: Histopathology of the Human Pulp.

61. R. Dubner: The Temporomandibular Joint and its Relationship to the Masticatory Musculature and the Occlusion of the Teeth.

62. R. O. Wolf and A. D. Merritt: Studies in Human Saliva.

63. A. D. Merritt and C. J. Witkop, Jr.: Genetics Studies in a Population Isolate (Brandywine) with Particular Reference to Hemoglobin and Haptoglobin Patterns.

64. A. D. Merritt and P. N. Baer: The Oral Glucose Tolerance Test in Patients With and Without Periodontal Disease.

CLINICAL INVESTIGATIONS: (continued)

65. A. D. Merritt: Studies of the Urinary Excretion Products of Histidine in Patients with Liver Disease.

66. A. D. Merritt and M. Kahn (NHI): Renal Function in Patients with Sjogrens Syndrome.

67. I. I. Ship: Investigations into the Etiology of Recurrent Aphthous Ulcerations and Other Oral Nonmalignant Soft Tissue Diseases.

68. I. I. Ship: Stress in the Dental Patient.

69. I. I. Ship: Secretory Sialography in Patients with Sjogren's Syndrome and Rheumatoid Arthritis.

70. I. I. Ship and O. Mickelsen (NIAMD): Effect of Dietary Phosphates on Dental Caries in Children.

Serial No. NIDR-48 (c)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Tri-racial Isolates in Eastern United States

Principal Investigator: Dr. C. J. Witkop, Jr.

Other Investigators: None

Cooperating Units: Calvin L. Beale, Agricultural Marketing Service, U. S. Department of Agriculture

Man Years (calendar year 1960):
Total: ¾
Professional: ¼
Other: ½

Project Description:

Objectives:

1. To determine the number, types, location and size of tri-racial isolates in Eastern United States.

2. To determine the degrees of inbreeding shown by various isolates.

3. To determine the amount and types of hereditary illness shown by the groups.

4. To verify or not the assumption that more individuals will show homozygous recessive hereditary illness in such populations.

5. To investigate the social consequences of isolate formation and their effects on the distribution and prevalence of intrinsic disease.

6. To investigate and compare the prevalence, types and inheritance patterns of intrinsic illness with in-group and out-group marriages, with degrees of consanguinity, with various other tri-racial groups, and with the general population.

Part A (continued)

7. In summary, the purpose of this investigation is to find, enumerate and describe all tri-racial isolates residing in Eastern United States; to investigate the degree of consanguinity in these groups; to look for unusual types of matings, rare in human material, that are of special value in determining modes of inheritance, gene interaction, and biochemical pathways; to investigate the hereditary diseases present in these groups; and to utilize the patient material found for further clinical and biochemical genetic investigations. It is assumed that in isolate populations, consanguinity will be prevalent; and it is further assumed that an increased number of individuals in such a population will show homozygous recessive illness. It is also assumed that all cases of a specific hereditary illness from any one group will nearly always be due to the same gene defect, thus providing an epidemiological method for selecting a large sample of individuals with the same gene defect for biochemical investigations in other NIDR projects in other Institutes.

Methods Employed:

This investigation requires three approaches:

1. Analysis of census and other civil records.

2. Survey of groups by letters to physicians and public health officials concerned.

3. Field work by NIDR teams.

a. Analysis of census and other records:

Because of the U. S. Census Bureau's wish to further classify people whose race entries were somewhat nebulous, the 1950 Enumerators Reference Manual directed, "Report persons of mixed white, Negro, and Indian ancestry living in certain communities in Eastern United States in terms of the name by which they are locally known". Such persons were then to be classified for publication purposes among "other nonwhite races," that is, other than Negro, American Indian, Chinese, Japanese, or Filipino. Starting with these data, and tracing the surnames so recorded through previous U. S. and British Colonial Census records, it was determined that such surnames frequently residing in the same household or neighborhood, showed variable race entries from decade to decade, even for the same individual (i.e., white, Negro, Indian or other), and for the most

Part A (continued)

part had large families. The existence of suspected tri-racial isolates from these data were then verified from local civil, historical, and social records such as marriage records, historical accounts, and newspaper articles. A search of the anthropological and social literature frequently revealed studies on such groups. Visits to these communities, to verify the existence and surname data, were made by collaborators and this investigator.

Of the 116 counties checked, the population of tri-racial character was estimated at 77,000 persons, of which 33,000 were listed as Indian, 29,000 as white, 14,000 as Negro and 1,000 under colloquial race names. Forty percent of the total reside in North Carolina.

Local and census records were used to estimate consanguinity and fertility. For example, census data from one North Carolina group showed the highest fertility ratio for any known racial or ethnic group in the United States. The ratio of children under 5 years old per 1,000 women, 15 to 49 years old, was 825 as compared to 417, the U. S. average. Marriage records of one group showed that 177 required ecclesiastical dispensation for relationship within the first three degrees (i.e., 2nd cousins or closer). Investigation of the marriage records in Barbour and Taylor Counties, West Virginia, showed that 102 of the 112 marriages from 1856 to 1931 were to other tri-racial group members. School records of the same group in 1958 showed an unusual proportion of students from the group in the higher age levels for the grade in which they were in.

b. Survey of groups by letters to physicians and public health officials:

After determining the number and surnames of persons comprising these isolate populations, letters were sent to all county health officials in the 116 counties where these people reside requesting information on specific listed hereditary illnesses among these groups and any illness that appeared unusually frequent in such populations. Other local physicians and dentists were also contacted. These returns were tabulated. Literature by all writers was scanned for mentioned hereditary illness in these groups (e.g., microphthalmia in the Moors of Delaware).

Part A (continued):

c. Field work by NIDR team:

It is planned to send investigators to these areas for 1-3 day preliminary reconnaissance work. If any important leads develop or if the reconnaissance shows promise of further study, these studies will be planned around the genetic material found. Some of this work has been done. For example, a visit to Halifax County, North Carolina, revealed a very rare hereditary eye lesion that has not, to my knowledge, been reported. A detailed clinical medical genetic study has been completed for the eye disease in this group.

Patient Material:

Patients found in this study of isolates have been admitted, from time to time, for the study of particular diseases.

Major Findings:

1. The reported high incidence of harelip and cleft palate in the Guinea group of West Virginia was not verified by our investigating team.

2. A hereditary form of benign intraepithelial dyskeratosis of mucosa and conjunctiva was found to affect 76 members of the Haliwar group in North Carolina. An unusual and undiagnosed hereditary neurological disease was also present in these people, associated with amino aciduria.

3. The reproductive rate of the Lumbee Indians of North Carolina is the highest of any ethnic group in the United States.

4. The size, location, distribution, emigration pattern, and a knowledge of the hereditary diseases present in many of these groups has been further described.

5. Several studies have been initiated on these groups by other investigators to whom we furnished the basic population data, as to location, surname, and population size. These studies have already shown several unusual hereditary conditions present in these groups, including hemoglobin disorders and sexual intermediates.

Significance to Dental Research:

These groups offer unusual genetic material for the study of oral, connective tissue, eye, and metabolic defects in sufficient quantities so that the geneticist can assure the clinical and

Part A (continued)

biochemical investigator that the genes involved are all of the same type. This offers an epidemiological method for the selection of patients with hereditary disease all with the same metabolic defect.

Proposed Course of Project:

To continue to accumulate information on the hereditary diseases present in these isolate populations.

Part B included: Yes

Serial No. NIDR-48 (c)

PHS-NIH
Individual Project Reports
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Beale, C. L. American Tri-racial Isolates; Their Status and Pertinence to Genetic Research. Eugenics Quarterly. 4:187-196, December 1957 (Published after 1957 project report)

Witkop, C. J., Jr., Shankle, C. H., Graham, J. B., Murray, M. R., Rucknagel, D. L., and Byerly, B. H. Hereditary Benign Intraepithelial Dyskeratosis. II. Oral Manifestations and Genetic Transmission. AMA Arch. Path. 70:696-707, December 1960

Honors and Awards Relating to this project: None

Serial No. NIDR-49 (c)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Hereditary Defects of Enamel and Dentin (Michigan Study)

Principal Investigator: Dr. C. J. Witkop, Jr.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):

Total: 1/4

Professional: 1/4

Other: 0

Project Description:

Objectives:

1. To define the various hereditary defects in enamel and dentin.

2. To determine the prevalence and mutation rates in the general population.

3. To determine the mode of inheritance.

4. To investigate the histological processes involved in the defects.

5. To describe any associated physical or chemical defect.

6. To investigate possible linkage associations.

7. To determine the various methods of restoration, prevention or treatment where possible.

Methods Employed:

1. Field Study. A survey of 96,761 school children, between the ages of 4 and 12, was conducted in 42 counties in the State of Michigan for hereditary defects and other formations.

Part A (continued)

Subsequent pedigree studied on these individuals were conducted to determine the mode of inheritance and possible linkage with known genetic markers, such as blood type, secretor factor, and P.T.C. taste test. These defects were classified by clinical descriptions, histological findings, and inheritance patterns. Patients with representative difficulties were brought to NIH for clinical evaluation.

2. Clinical Study. Complete physical and routine laboratory examinations were given to determine if any physical or metabolic defect was also associated with the tooth defect. Except in one particular defect, osteogenesis imperfecta, no genetic defect was found associated with the tooth condition.

3. Laboratory Study. Histochemical studies conducted on fresh ground and decalcified specimens using normal controls, showed that there was a possible biochemical defect in opalescent dentin. Linkage studies were analyzed.

Patient Material:

Patient material was obtained from 1) individuals contacted during our field study in Michigan; 2) individuals in our Genetic Study in Southern Maryland, Project No. D-4-002; and 3) in a few instances from individuals referred by local practitioners.

Major Findings:

1. The mutation rate for opalescent dentin is extremely low. No case that could be a new mutation was found among the 42 propositi in this study. A check of other cases reported in the literature and by other investigators failed to find a single instance of this disease that could possibly have arisen by mutation. This is the only autosomal dominant hereditary condition in man in which a mutant has never been found.

2. The homozygous state of opalescent dentin may be lethal causing intrauterine deaths.

3. From a comparison of the Michigan population with the Maryland population, one of two possibilities exists. Either the defect known as opalescent dentin is the same

Part A (continued)

in both populations, with the gene manifesting more severe symptoms because it is present in a different gene complex in the Maryland population; or the gene is different in the Maryland population and, therefore, a different disease. If the genes are the same, the significance would indicate that defective genes adapt over many generations to the rest of the gene complex, and when put into a stock with different racial background, manifest themselves in a more severe form.

4. Some evidence is present that a bone defect of a mild type is found in people with opalescent dentin.

5. Found during this survey was a hereditary, tumor-like condition of the teeth closely associated with dens-in-dente, which is also probably hereditary, and with odontomas, which have been described as dentin dysplasia. This is inherited as an autosomal dominant trait.

6. Dens-in-dente shows a familial pattern probably inherited as a dominant trait.

7. We have found a hereditary condition consisting of premature closures of the fontanels, ptosis of the eyelid, absence of tear ducts, and missing lateral incisors that is inherited as an autosomal condition. These individuals require prompt surgical intervention for the cranial defect. Many also need surgical correction of an associated agenesis of the condyles of the mandible.

Significance to Dental Research:

The etiology of several conditions may now be ascribed to hereditary causes. The clinical description of the oral defects has been given, and experience in surgical correction and restoration has been obtained.

Proposed Course of Project:

We recently have initiated, with Dr. Karl Piez, NIDR, an investigation to try to determine the biochemical defect present in opalescent dentin. Histochemical studies indicate that there are more chemically reactive groups in the matrix of the dentin in the affected teeth than in normal teeth. A basophilic P.A.S. positive substance is present in dentinogenesis imperfecta similar to that found in the bone of osteogenesis imperfecta. We believe that this is ar abnormal protein. The chemical methods are being perfected for analysis of normal bone, dentin, skin, and tendon,

Part A (continued)

which will be utilized to investigate a variety of hereditary defects. The first of these will be opalescent dentin. Continued accumulation of pedigree material and linkage data is planned.

Part B included Yes

Serial No. NIDR-49 (c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Witkop, C. J., Jr.: Genetics and Dentistry: Eugenics Quarterly, 5:15-21, March, 1958

2. Witkop, C. J., Jr.: Genetics and Dentistry: (in Hammons, Helen, G. ed. Hereditary Counseling Hoeber, N. Y., 1959) pp. 13-28

3. Witkop, C. J., Jr: Dental Genetics 1959, J.A.D.A. 60:564-576, May, 1960

Honors and awards relating to this project:

None

Serial No. NIDR-50 (c)
1. Clinical Investigations
2. Human Genetics.
3. Brandywine, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Genetic Study in Southern Maryland

Principal Investigator: Dr. C. J. Witkop, Jr.

Other Investigators: Dr. L. J. Schneiderman
Dr. R. O. Wolf
H. R. Dyson, R.N.
Dr. A. Donald Merritt
Dr. B. Hanna

Cooperating Units: Blood Bank DBS; School of Dentistry, Howard University; Sociology Department, Catholic University.

Man Years (calendar year 1960):

Total: 5

Professional: 1½

Other: 3½

Project Description:

Objectives:

1. To determine the total amount of hereditary illness present in an isolate population of not over 5,000 related individuals of white, American Indian, and Negro ancestry as revealed by dental and physical examinations and selected laboratory procedures.

2. To determine the prevalence, mode of inheritance, effects on viability and longevity, clinical manifestations and variations in expressivity of the hereditary illnesses found.

3. To investigate the relationships and possible linkages between these illnesses and blood types, secretor factor, P.T.C. taste test and other genetic markers.

Part A (continued)

4. To investigate genetic factors in illness not usually known to have a hereditary component.

5. To select patient and biological material from this population for clinical and biochemical investigations of specific illnesses and normal factors (these will constitute separate projects).

6. To assess the effects of inbreeding as revealed by in-group and out-group marriages and by degree of relationship (i.e., first cousins, first cousins once removed, second cousins, etc.) as shown by perinatal deaths, viability, longevity, and the presence of apparent homozygous recessive illness.

7. To try to trace the source of outflow of pathological genes in this population.

8. To assess the social and genetic factors in mate selection.

9. To look for correlations between genetic illnesses and other normal and abnormal factors that may have etiological or diagnostic relationships.

10. To try to reconstruct, on the basis of gene frequencies, the sources of the group's genetic material.

11. To compare the prevalence of illness, gene frequencies, and vital statistics with other isolate populations and the general population.

12. To detect carriers of recessive genes, if possible.

Methods Employed:

This study is divided into three phases:

1. Field study:

The field study consists of history and dental, medical and laboratory examinations designed to detect as much hereditary illness as possible.

a. History, genetic and census data are obtained on a questionnaire covering 158 items which arranges the data by family groups and by sibships. Such items as birth order effects, parental age, consanguinity, age of onset, longevity, and perinatal

Part A (continued)

mortality are included. A record of hospitalizations and institutionalizations is obtained in addition to a medical and genetic history. A master kindred chart showing the relationship of each individual is made.

b. A search of existing social and church records for births and marriages is made by Fr. Thomas Harte of Catholic University. Sociological data on development in marriage patterns in this group are also obtained.

c. All subjects in this study are given a complete dental and medical examination, designed to detect as much hereditary illness as possible. This includes complete blood typing genetic markers; urine analysis; hematological work-up, expecially for abnormal hemoglobins; and special procedures such as X-rays and ophthalmological examinations where indicated. These examinations are made during the summer months at mobile units.

d. All data are coded and tabulated for I.B.M. punch cards.

2. Clinical Study:

Selected patients seen in the field study are brought to NIH for detailed clinical studies. These studies are designed to give a description of the hereditary diseases involved; to determine the limits of the signs and symptoms of the illness; and to describe any metabolic abnormalities detectable on routine laboratory examinations, or other associated defects that might be found on X-rays or by special procedures.

3. Biochemical Investigations:

Selected genetic defects are being investigated biochemically to find the chemical error present. Currently under study are sickle cell disease, dentinogenesis imperfecta, and diffuse goiter. The sickle cell disease is being investigated by tonometry, electrophoresis, and the survival of this abnormal hemoglobin in transfused patients. Dentinogenesis imperfecta is being investigated histochemically and by chemical analysis for abnormal protein constituents. The goiter patients are being studied for the presence or absence of non-thyroglobulin thyroid proteins, the response to thyroid stimulating hormone, serum electrophoresis,

Part A (continued)

total protein bound and butanol extractable iodide, and incubation of thyroid biopsies with I^{131}.

Patient Material:

Patient material is selected from the families seen in the field studies. Approximately 500 hospital days have been utilized by this and other institutes.

Major Findings:

These data have not been analyzed statistically; however, a preliminary examination indicated the following:

1. There appears to be a hereditary block in thyroglobulin synthesis that accounts for the prevalence of goiter in about 8 percent of the women in this group.

2. A new hereditary bone disease, best described as generalized hereditary osseous hyperplasia, has been found in this group to be inherited as a recessive trait, and is known to exist in three other families. This is a newly described bone condition in which all deaths, with one exception, of people showing this disease have been attributed to respiratory causes.

3. Ankyloglossia appears to be inherited as a dominant trait.

4. There appears to be a genetic basis for certain speech defects not necessarily associated with gross oral abnormalities.

5. Four patients with a rare blood type, which has a frequency of .00008 in the general population, have been found.

6. Genetic evidence has been obtained to show that either there is an alternate pathway in melanin synthesis (other than that presently proposed) or that a secondary pigment system is present in humans.

7. A familial form of gynecomastia exists that is not Klinefelter's syndrome.

8. The frequencies of dentinogenesis imperfecta and albinism in the Brandywine group are the highest known in any population.

Part A (continued)

9. The frequency of sicklers of 20.5% is the highest outside Africa. Preliminary analysis of these data show no liability for the SA gene type.

Significance to Dental Research:

An isolate population has been found containing a vast number of hereditary diseases which may shed light on genetics and biochemical pathways of various hereditary defects.

Proposed Course of Project:

Data are being processed for machine analysis.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Dyson, Hazel R., Witkop, C. J., Jr., and Butters, Shirley S. Genetics in the Public Health Nursing. Eugenics Quarterly. 5:23-28, March 1958.

Carter, J. F. A Description of Some Speech Anomalies in a Tri-racial Isolate Group. A thesis, presented in partial fulfillment of the requirements for a Master of Science Degree, May 1958, University of Maryland.

Harte, T. J. The Use of Parish Records in Social Research. Am. Catholic Sociological Review, June 1958.

Harte, T. J. Trends in Mate Selection in a Tri-racial Isolate. Social Forces, November 1958.

Dyson, H. R., Witkop, C. J., Jr., and Butters, S. S. Genetics in Public Health Nursing. Chap. 3 (In Hammon, H., ed. Hereditary Counseling, Hoeber, N. Y., 1959) pp. 29-41.

Witkop, C. J., Jr. Tyrosinase and Albinism. Am. J. Human Genetics. 11:91, March 1959.

Hursey, R. J., Witkop, C. J., Jr., Miklasheck, D., and Sackett, L. M. Dentinogenesis Imperfecta in a Racial Isolate with Multiple Hereditary Defects. Oral Surg., Oral Med., Oral Path. 9:641-658, June 1956

Witkop, C. J., Jr., Dyson, H. R., and Sackett, L. M. Hereditary Defects Occurring in a Racial Isolate Residing in Southern Maryland. Clin. Proc. Child. Hosp. Washington, D.C. 12:29-33, 1956

Honors and awards relating to this project: None

Serial No. NIDR-51 (c)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Sickle Cell Anemias, Other Inherited Hematological Disorders, and Blood Groups

Principal Investigator: Dr. C. J. Witkop, Jr.

Other Investigators: Dr. D. L. Rucknagel
Dr. L. J. Schneiderman
Dr. Robert O. Wolf
Dr. A. D. Merritt

Cooperating Units: Department Human Genetics, University of Michigan
Division of Biologics Standards

Man Years (calendar year 1960):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives:

To study, in detail, physiologic, biochemical, and genetic factors in various inherited hematologic disorders in the Brandywine tri-racial isolate. Laboratory and clinical data will be integrated with the pedigree and analyzed for the purpose of defining biochemical mechanisms and mode of inheritance of disease. In addition, prevalence figures for abnormal hemoglobins will be determined in various surveys in which the Section participates. These include the ICNND Nutritional Survey of Chile, various North Carolina Groups, and American Indians.

Methods Employed:

Field surveys among the Wesort groups have detected individuals with several hematologic disorders. Approximately twenty percent of this group harbor the sickle cell gene. Individuals with sickle cell anemia are hospitalized and evaluated by standard hematologic procedures. In addition, specialized

Part A (continued)

investigations of intravascular sickling, arterial oxyhemoglobin saturation, and erythrocyte physiology using tonometric techniques are utilized.

Relatives of propositi are studied hematologically using standard hematologic techniques, paper and starch block electrophoresis and, when indicated, oxygenation kinetic studies.

Non-sicklemic anemic individuals and their families are also being studied to elucidate other genetic abnormalities. One individual with pernicious anemia was studied extensively (in cooperation with Drs. Seegmiller, LaDu and Laster) in an attempt to elucidate the effect of vitamin B_{12} deficiency upon purine biosynthesis. The prevalence of blood factors and the correlation with genetic disease and nutritional status in various populations was made for blood type, red cell enzymes and abnormal hemoglobin.

Patient Material:

This year another 450 individuals were screened in the Brandywine field examinations. Anemic individuals were selected for the most part from this group. A few non-group sickle cell anemic patients were studied as control subjects. Fetal hemoglobin assays on blood of patients with choriocarcinoma are continuing, and additional studies are also under way on patients with an assortment of other malignancies being treated by the National Cancer Institute.

In Chile 1906 Chileans residing from Punta Arenas to Arica were examined for dental defects and nutritional status. Of these individuals, 673 were blood typed; the erythrocytes were examined for glucose-6-phosphate dehydrogenase activity; and the hemoglobins were analyzed by paper electrophoresis for abnormalities. Samples from 120 persons of a tri-racial isolate in North Carolina were screened for abnormal hemoglobins and blood type. Three hundred North American Indians and 140 Araucanian Indians of Chile were surveyed.

Major Findings:

1. During the past year serial clinical studies and erythrocyte oxygenation investigations have been performed on eighteen individuals with sickle cell anemia. These have indicated that the amount of intravascular sickling and the degree of arterial

Part A (continued)

oxygen unsaturation are reasonably constant in a given individual over a long period of time. Quantitative studies have documented several degrees of severity of these disease manifestations. Moreover, laboratory investigations have demonstrated that variations in clinical manifestations and in arterial saturation can be correlated with abnormalities in erythrocyte physiology per se, and not with associated systemic disease, i.e., pulmonary insufficiency, cardiac anomalies, etc.

2. The patient with pernicious anemia was found to excrete large amounts of formimino glutamic acid, a compound involved in folic acid metabolism, since it is also excreted by rats and humans receiving the folic acid antimetabolite, amethopterin. In addition, carbon-14-labelled formamidino aminoimidazole carboxamide was incorporated less efficiently into uric acid than aminoimidazole carboxamide in our patient. Both of these observations implicate defective formyl group metabolism in pernicious anemia.

3. To date, increased amounts of fetal hemoglobin have been found in 10 of 35 patients with choriocarcinoma studied. Bloods from 30 individuals with other malignancies contained normal amounts of fetal hemoglobin. Since chorionic gonadotropin is considered the stimulus to fetal hemoglobin production in these patients, it may also increase the fetal hemoglobin content of cells of patients with sickle cell anemia, thereby alleviating the clinical severity. However, preliminary trials on a small number of sickle cell anemia patients have shown no significant results to date.

4. The prevalence of abnormal hemoglobins in Chileans was low. Two patients with SA and one with AC hemoglobin were found in 673 tested. No examples of low glucose-6- phosphate dehydrogenase activity were found.

5. The Araucanian Indians tested had no abnormal hemoglobins or glucose-6-phosphate dehydrogenase activity.

6. The gene frequencies of Chileans and Araucanian Indians was determined for ABO, Rh, MN, Kell, Duffy, Lewis, and other blood types. These indicate a considerable amount of admixture of Indian in the Chilean population. The fourth known example of blood type RhG was

Part A (continued)

found in this sample.

7. Inheritance patterns in 126 large families for haptoglobin have been determined.

Significance to Dental Research:

Since it is recognized that genetic factors are important in the study of dental diseases as well as other medical problems, this program is aimed at evaluating genetic mechanisms by as many approaches as are feasible. By studying specific entities as thoroughly as possible and correlating variations of disease and laboratory findings with pedigree data, it is anticipated that conclusions may be forthcoming regarding genetic mechanisms.

Proposed Course of Projects:

Enlargement of the pedigree will continue but hematologic investigations are now being concentrated in areas of the pedigree deemed to be most promising. These are designed to elucidate the presence and nature of modifying hereditary factors in sickle cell anemia, and to evaluate the presence of Thalassemic genes in the non-sicklemic anemia sibships. Data are now being processed for I.B.M. machine analysis.

Other specific projects planned include:

None: Analysis of data and publication

Part B included No

Serial No. NIDR-52 (c)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Oral Aspects of the Results of Consanguineous Marriage in Hiroshima and Nagasaki, Japan

Principal Investigator: Dr. J. D. Niswander

Other Investigators: Dr. C. J. Witkop, Jr.

Cooperating Units: National Academy of Sciences, Washington, D. C.; University of Michigan Department of Human Genetics under the direction of James V. Neel, William J. Schull, and James N. Spuhler

Man Years (calendar year 1960):

Total: 1

Professional: 1

Other: None

Project Description:

Objectives:

1. In connection with the genetic program of the Atomic Bomb Casualty Commission in Hiroshima and Nagasaki, Japan, the details of which have been presented by Neel and Schull (Neel, J. V. and Schull, W. J.: The Effect of Exposure to the Atomic Bombs on Pregnancy Termination in Hiroshima and Nagasaki. National Academy of Sciences, #461, Washington, 1956), the outcome of pregnancy was determined in 5,033 instances in which the parents reported consanguinity of some degree. The products of these pregnancies would, if now alive, range in age between five and ten years at this time. In essence, this proposal involves an attempt to obtain a detailed follow-up study on each of these 5,033 pregnancy terminations, plus a suitable control material.

2. Of the 5,033 pregnancy terminations, a number are known to have resulted in stillbirths or deaths during the first nine months of life. Starting from the records of the Atomic Bomb Casualty Commission, a trained staff of

Part A (continued)

"field workers" will attempt to re-establish contacts with all of the remaining children (i.e., registered in the Genetics Program and not known to have died during the period of time covered by this program). This attempt will result in a roster of living children, a roster of now-deceased children, and a roster of children who have moved from the two study cities. An effort will be made to establish cause of death for all deceased children. The background of the children who have moved from the cities will be analyzed, in a search for biasing factors. It is possible that an attempt will be made to contact these latter children with reference to the question of present viability. The fact of death and, possibly, the cause of death will be analyzed in relation to parental consanguinity.

3. A pilot study carried out in 1956 under the direction of W. J. Schull has already revealed that the death rate among the children of consanguineous marriages is approximately twice the control death rate (cf. Appendix). There is, then, already considerable assurance of significant results from this inquiry. These same data can be analyzed in relation to the radiation history of the parents, thus yielding additional information concerning the genetic effects of the atomic bombs.

4. The dental portion of this study proposes to determine the effects of consanguinity on DMF rates, periodontal disease indices, growth and development problems, and specific hereditary diseases affecting the oral cavity.

Methods Employed:

Using ABCC facilities in Nagasaki and Hiroshima, a team consisting of specialists in such fields as pediatrics, hematology, dentistry, and anthropology began examinations of offspring of consanguineous marriages and appropriate control groups in September 1958. The following examinations were performed:

1. Physical examination: This consists of a standard physical examination, with emphasis on characteristics which can be graded in an objective manner lending itself to statistical analysis.

   a. Presence of gross physical defect; i.e., abnormalities of skin, musculature, skeleton, etc.

Part A (continued)

b. Defects of the auditory mechanism, with special reference to loss of hearing.

c. Defects of the eye, including loss of visual acuity.

d. Cardiac abnormality (cardiomegaly, disorders of rhythm, murmurs, etc.).

e. Pulmonary abnormality.

f. Apparent abnormality of abdominal viscera.

g. Abnormality of external genitalia.

h. Neurological evaluation.

i. Bone age (single wrist film).

j. Determination of leucocyte count and hemoglobin level.

k. Standard urinalysis.

2. The dental examination includes pathology of the soft tissues, pathology of the teeth and index of malocclusion, index of tooth loss, developmental failures of oral structures, DEF and def rates, and the association of these conditions with and without generalized hereditary disease.

3. Anthropometric examination: This will include such standard items as weight, sitting and standing height, span, measurements of head and face, subcutaneous fat index, etc.

4. Psychometrics: Each child was tested at time of clinic visit by means of a portion of the WISC test (maze and trail). In addition the complete Japanese standardized WISC was administered by the Hiroshima University Department of Psychology.

5. School performance: Access was obtained to school records for each child and selected information abstracted.

All results will be coded and transferred to IBM cards for statistical analysis. As for the Genetics Program data, the statistical analysis will undoubtedly draw heavily on an approach which makes allowance for certain uncontrollable sources of bias. The first result will be a comparison of the different groups of children with respect to a wide

Part A (continued)

variety of characteristics with particular reference to the regression of the findings on degree of inbreeding. Beyond this, however, it is anticipated that the material will permit tentative conclusions concerning the number of "lethal-equivalents" carried by the average Japanese, utilizing the mathematical approach suggested by Morton, Crow, and Muller (1956).

Major Findings:

The field work in Japan was completed in August 1960. Final accounting of the study group is presented in the following tables:

Final CHS Records Report, Hiroshima:

	Related	Unrelated	Total
Potential study records	1815	1981	3796
Less stillborn	67	52	119
Less moved	323	382	705
Less not traced	13	7	20
Less dead (family in city)	131	98	229
Less refusals	26	35	61
Clinic examinations	1255	1407	2662

Final Accounting of CHS Regular Study Cases, Nagasaki:

	Related	Unrelated	Total
Potential cases	2783	2805	5588
Deceased	186	168	354
Moved	385	446	831
Refusals	13	11	24
Not traced	1	1	2
Stillbirths	71	85	156
Clinic examinations	2127	2094	4221

Part A (continued)

Data collected in Japan are being assembled at the Department of Human Genetics, University of Michigan. Preliminary analysis will begin about December 1960. First stages will consist of checking for internal consistency of the data and for sources of bias within and between the study groups. It is anticipated that analysis for specific inbreeding effects will begin early in 1961. From normative data on Japanese children of Hiroshima, it has been determined that between 1950 and 1958, for 10-year-olds, there has been an average of 3.35 cm. increase in stature. Between 1951 and 1959 there were significant increases in stature, weight and number of permanent teeth erupted for 6, 8, and 10 year old children.

Frequency of supernumerary teeth was observed to be higher than has been reported for Caucasians (about 3%) and a striking sex difference noted (4% in males and 2% in females). Racial differences were also noted in the frequency and distribution of congenitally missing teeth. An additional observation of dental interest is an apparent relationship between caries of the deciduous teeth and structural defects in the enamel of the permanent successors.

Significance to Dental Research:

1. Properly executed studies on consanguinity effects will yield information on the genetic structure of human populations which at present can be obtained in no other way. Such information is vital to a realistic, quantitative appraisal of such problems as are posed by the increasing exposure of the human species to ionizing radiation.

2. The Japanese Study will offer an opportunity to study the genetic effects of a specific type of inbreeding, complementing our Brandywine Study. Whereas, the latter program is assessing the effects of continuous inbreeding, the Japanese Study has the unique advantage of affording an evaluation of one type of mating pattern.

3. It would be expected that certain recessive characteristics determining normal growth and development, as well as pathological traits, would show up in the homozygous state with increased frequency. Many of these conditions are probably unknown, and the Japanese Study consequently offers an opportunity to define both normal factors and pathological traits.

Part A (continued)

4. The Brandywine population group is descended from relatively few ancestors. Consequently, unless an ancestor carried a hidden trait it could not appear in the descendants, that is, barring mutation. Therefore, the Brandywine group would present only those inherited conditions carried by predecessors. On the other hand, the first cousins in Japan are drawn from a large unrelated population and, therefore, many characteristics and pathological traits should appear in their offspring.

5. In addition to the study of inbreeding effects, the normative aspects of the data offer numerous opportunities for the investigation of the role of racial and environmental factors in many oral conditions.

Proposed Course of Project:

Analysis of inbreeding effects is expected to require approximately two years.

Part B included: Yes

Serial No. NIDR-52 (c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Niswander, J. D., and Sujaku, C. Dental Eruption, Stature, and Weight of Hiroshima Children. J. Dent. Res. 39:959-963, September-October 1960.

Schull, W. J., and Neel, J. V. The Child Health Survey: A Genetic Study in Japan. WHO Seminar on the Use of Vital and Health Statistics for Genetic and Radiation Studies, Geneva, 1960 (In press).

Honors and Awards Relating to this Project: None

Serial No. NIDR-53 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies of the Oral Microbiota and Oral Environment in Relation to Dental and Systemic Diseases.

Principal Investigator: Dr. R. M. Stephan

Other Investigators: None

Cooperating Units: Dental Clinic, Clinical Center, NIH

Man Years (calendar year 1960)
Total: 1½
Professional: ½
Other: 1

Project Description:

Objectives:

1. To study macroscopically and microscopically the occurrence, growth, localization, morphology, physical characteristics, and color reactions of microbic plaques on the teeth, in gingival crevices, in periodontal pockets, and on the oral mucous membranes.

2. To determine differences in microbic plaque formation, food retention, salivary flow rates, gingival exudates, and pH levels which are associated with caries, erosion, periodontal disease and xerostomia in comparison with normal oral conditions.

3. To determine specific types of microorganisms in plaques using phase microscopy, fluorescent antibody techniques, micro culture slides, and selective media.

4. To investigate possible associations of specific types of oral microorganisms with dental and systemic diseases.

Methods Employed:

Direct stereomicroscopic observations in the oral cavity are made at 6X-40X magnifications with the Zeiss "Otoscope." Direct pH measurements are made with micro antimony and glass electrodes and

Part A (continued)

with microapplicators containing colorimetric indicators. Measurements of parotid salivary flow rates are made with the "Lashley Cup" apparatus. Samples of plaques and other material from the teeth, periodontal pockets, and mucous membranes are taken with dental explorers, scalers, swabs, and specially designed microscalers for quantitative samples which can be weighed on a microbalance. These samples are studied in micro culture slides by phase microscopy, in smear preparations using the fluorescent antibody techniques to label specific microorganisms, in gram stained smears, and in selective culture media.

Patient Material:

Eighty-eight patients have been studied this year. Most have been outpatients of NIDR referred for study of rampant caries, dental erosion, severe periodontal disease, or stomatitis. Patients with various diseases who have been referred from the other Institutes of NIH have been studied for possible interrelationships between oral conditions and other diseases (e.g. Sjögrens Syndrome, in collaboration with NIAMD).

Major Findings:

1. Further information has been gained on the complex hostparasite relationships in the oral cavity, particularly as they relate to caries, erosion, periodontal disease and xerostomia. Quantitative estimates of the different forms of organisms in surface material removed from representative localized areas of the oral environment have demonstrated wide variations in the microbiota between different individuals, and between different areas in the same individual. At the same time, a more or less basic underlying pattern has been found for the distribution of certain common types of organisms, such as spirochetes in gingival crevices and streptococci on tooth surfaces. The most interesting findings have been the unusually heavy growth of microbic plaques in rampant caries and the presence of many different forms of motile organisms in severe periodontal disease. For example, growth of Endamoeba gingivalis and Trichomonas tenax in periodontal pockets has been observed by phase microscopy and by culture in egg media. However some other forms have not yet been grown in pure culture nor identified in histological material. Thus, their importance and general distribution is not well understood.

2. Studies on intra-oral pH of plaques have continued to show normal pH levels between 6.0 and 8.0. In active caries, pH levels of plaques have been as low as 4.2. pH levels as low as 5.4 have been found in an unusual type of nonadherent "plaques"

Part A (continued)

in active dental erosion areas, while most erosion areas take on the same pH as the saliva.

3. The salivary rate of flow has been measured in patients with Sjögrens Syndrome and with arthritis in cooperation with Dr. Bloch, NIAMD. A significantly lower flow rate was found in patients with Sjogrens Syndrome than in normal controls.

4. Patients who have received therapeutic X-ray irradiation of the head and neck have been of particular interest since, in some cases, they develop a lack of oral secretions which is usually associated with highly rampant dental caries.

Significance to Dental Research:

This project has been planned to cover a neglected area in oral pathology and micro-biology - namely, to furnish information on the total microbiota in the oral cavity as it grows "in situ" and as it is related to clinical disease processes.

Proposed Course of Project:

It is planned to continue clinical studies on rampant caries and dental erosion, and particularly to develop the use of the fluorescent antibody technique for specific organisms in dental plaques which may be characteristic of rampant caries. Much useful information on the dental plaque has been produced, and it is planned to complete statistical analysis of the data.

Part B included: No

Serial No. NIDR-54 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Interrelationships Between Local and Systemic Factors in the Etiology of Dental Disease in Experimental Animals.

Principal Investigator: Dr. R. M. Stephan

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1¼
Professional: ¼
Other: 1

Project Description:

Objectives:

1. To determine the essential experimental conditions which control the development of dental caries and periodontal disease in experimental animals.

2. To study the etiological relationships between specific oral bacteria and bacterial plaque, tooth formation, food retention, intra-oral pH, and the development of caries.

3. To develop the use of vital stains and fluorescent dyes as indicators for the earliest stages of caries.

4. To explore the caries-inhibiting action of some antibacterial dyes and antibiotics which can be deposited in combination with calcified tooth substance.

Methods Employed:

1. Four basic diets have been used: (a) a nutritionally defined diet of sucrose, casein, salts, and known vitamins which induces very extreme caries, (b) a nutritionally defined diet which induces little caries, (c) a stock diet which does not induce caries, and (d) Keyes' cariogenic diet for hamsters (Project No. 34).

Part A (continued)

2. In multifactorial experiments, rats and hamsters have been inoculated with cariogenic bacteria received from Drs. Fitzgerald and Keyes (Project No.34) and cultures from clinical cases of rampant caries. Diets have been supplemented with antibacterial and nutritional substances to determine systemic effects and the relation of growth rates and oral flora to caries development.

3. Glucose and sucrose have been added to the drinking water to produce both local oral and systemic effects.

4. Vital stains have been injected intraperitoneally, added to the diet, or added to the drinking water to serve as indicators for the early stages of caries. Stains have also been studied as indicators for pH and caries activity, and fluorescent effects of some stains and antibiotics have been observed in teeth using ultraviolet illumination under the stereomicroscope.

5. Bacterial plaque formation has been studied microscopically on the incisor teeth in the live animal, and direct pH measurements of the plaques have been made with micro-antimony and glass electrodes and with pH indicators.

Major Findings:

1. Further observations have been made on a number of vital stains which have been found to be good indicators for the initial stages of caries. One of the most interesting findings is that some dyes stain only the very earliest stages of caries in enamel whereas others stain only the advanced stages of caries. Although the hydroxyanthraquinone dyes continue to yield the best stain for bones and teeth, dyes from the oxazin and xanthene groups have been found useful for staining caries. There are several basic chemical reactions which can be demonstrated in the caries process by improved vital staining methods. Ultraviolet illumination has revealed fluorescent staining with substances such as aureomycin which combine with calcified tooth substance but have too little color to be visualized by ordinary illumination.

2. The effect of additions of sucrose and dextrose to the diet and drinking water has greatly increased caries in some experiments, and only moderately increased it in others. This has been shown to be due partly to the age of the animal and to the fact that when sugar is given early, the effect on caries is greatest.

3. Attempts to increase caries in rats and hamsters by inoculating with bacteria from the mouth of people with rampant caries have not succeeded. The finding of Dr. Keyes that caries can be induced in albino hamsters by inoculation with certain bacteria has been reproduced, but there is still considerable variation in the susceptibility of individual animals. These bacteria have not induced caries in rats.

4. These studies have continued to demonstrate that the development of caries results from the interaction of many different factors including the strain of animal, the physical properties of the diet, the nutritional value of the diet, the retention of diet in the oral cavity, the growth of microorganisms on the teeth, and the relative growth and activity of the individual animal.

Significance to Dental Research:

The development of improved methods for studying the etiology of dental caries and periodontal disease in laboratory animals will permit more precise testing of the relative importance of many factors which may potentiate or control these diseases.

Proposed Course of Project:

It is clear that both dental caries and periodontal disease are very complex problems and that additional new methods are needed for determining their activity. It is planned to continue multi-factorial experiments in animals to further delineate the inter-relationships of different types of factors in the development of dental diseases, and particularly to explain further the possibility of testing the cariogenicity of oral bacteria from humans by inoculation in experimental animals.

Part B included: No

Serial No. NIDR-55 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: General Anesthesia (Dental) Baseline Data

Principal Investigator: Dr. E.J. Driscoll

Other Investigators: Dr. G. Christenson and Dr. C. Herbert

Cooperating Units: Anesthesiology Department, Clinical Center

Man Years (calendar year 1960):

Total:	1
Professional:	3/4
Other:	1/4

Project Description:

Objectives:

It is estimated that in many parts of the country, particularly in the East and far West, there are nearly as many general anesthetics administered in dental offices as there are in the local hospitals.

1. Dental general anesthesia is different from ordinary hospital anesthesias in several important respects: (a) the patients are ambulatory and not usually sedated; (b) the operations are performed in as light a plane as possible; (c) the operations are performed in or about the airway; and (d) the patients expect to be incapacitated, only for short periods of time because of these major differences, the background of knowledge in general dental anesthesia is incomplete and not accurate or comparable. Thus, a great need exists for fundamental data concerning alterations in the physiological mechanisms, and a definition of the limits beyond which hazards are faced.

2. The accumulated data from this study has been and will continue to be used as a baseline of comparison for the many new anesthetic drugs which have been and are being proposed for use in oral surgery.

Methods Employed:

1. For the purpose of standardization of surgical trauma, full mouth extractions have been utilized rather than random oral surgical cases. The teeth are extracted in quadrants and the entire operation systematized for time of procedure and traumatic experience. Under these conditions, the following agents have been undergoing evaluation.

(a) Sodium methahexatol, Pentathal and Neraval.

(b) The parasympatholytic agents atropine, scopalamine and hexocyclium barbiturates with nitrous oxide-oxygen.

2. Physiologic data being recorded are pulse, blood pressure, arterial O_2 saturation, respiratory phenomena, cortical brain activity and the electrical activity of the heart (E.K.G.). Likewise recorded are such important technical data as amount of drug used in units of time, length of operation, duration of sleep, mental alertness on awakening, age, race, sex, and emotional status in relation to the conduct of anesthesia, and post-anesthetic sequelae (headache, nausea and vomiting, depression and hiccoughs).

Patient Material:

This year another 150 detailed studies were completed on about 50 adult patients.

Major Findings

1. Sufficient data is now available to determine objectively from brain wave activity the precise anesthetic levels at which various procedures are being carried out with the three barbiturates Pentothal, Neraval, and Methahexitol. The addition of N_2O and O_2 does not significantly alter these E.G. patterns, nor does short periods of apnea, as some investigators have indicated in the literature. Thus, it is definitely established that we are working in an extremely light plane.

2. The E.E.G. is also making it possible for us to state with certainty the precise anesthetic plane which is present at specific intervals when other physiologic measurements such as pulse and pressure are made and studied in relation to trauma.

3. Both atropine and Tral reduced the number of arrythmias, as Banthine had done in our previous study. Both of these agents were given before the administration of the barbiturate. Atropine alone was found to accelerate the pulse within a minute whereas Hexocyclium had no such accelerating property until the barbiturate was added.

However, when the barbiturate was given, the acceleration caused by both Tral and atropine was comparable.

4. Hexocyclium was found to be a far superior drying agent than either atropine or scopalamine. Another practical feature about hexocyclium as compared to atropine and scopalamine is that its action is of short duration; i.e. it clinically reduces secretions very effectively for 20 minutes. Thus, in the post-operative period when the patient becomes oriented, there is no annoying problem of a dry mouth.

5. The significant blood pressure elevation and pulse acceleration continues to be our most important finding from a practical standpoint. It was found that the blood pressure and pulse elevation with Methahexitol was slightly greater than that we reported with Pentothal and Neraval. Although the values are about 10% greater for both pulse and blood pressure, the pattern of rise is identical with the two previously described drugs, i.e., the pulse peaks in one to two minutes whereas the blood pressure peaks in six minutes indicating a slightly different physiological mechanism in operation in these closely allied physiologic measurements.

Significance to Dental Research:

1. The search is constantly being made in dentistry for safer general anesthetic agents. However, without baseline data on physiologic responses, this search has no foundation. Methahexitol is a good example of the new drugs which have been recently introduced, and our studies on this drug demonstrate the value of our previously accumulated data on intravenous barbiturates as far as baselines are concerned. With our previously acquired baselines on Pentothal and Neraval, we are in a good position to evaluate not only Methahexitol but any other new anesthetic drug which may be introduced for use in ambulatory oral surgery.

2. The possibility also exists for improvement and refinement of present methods of anesthesia as well as laying a sound foundation for further anesthesiology research. An example of refinement in methods is indicated in our studies of N_2O and O_2 supplementation and demerol premedication, both of which help to control adverse rises in blood pressure and pulse.

Proposed Course of Project:

1. The study will continue on both good risk (ASOS class I) and medium risk (ASOS class II) outpatients. These medium risk patients have nothing wrong with their cardiovascular or respiratory systems which would make them unsuitable candidates for general anesthesia.

Additional baseline and comparative data will be accumulated, studied, and evaluated.

2. The study of Methahexitol will continue, especially studies in which various concentrations of N_2O and O_2 are used as supplements.

3. The effects of the vagolytic agents will be further recorded and observed. Studies of arterial oxygen saturation by use of the air oximeter has been found to be very effective and will be continued.

4. Studies on continuously recorded respiratory data have been included for the first time in this years studies, and they will be continued.

Part B included: Yes

Serial No. NIDR-55 (c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Driscoll, E.J., and Christenson, G.R. and White, C.L. Physiological studies in general anesthesia on ambulatory dental patients. O.S. O.M. and O.P. December 1959.

2. Christenson, G.R., Hebert, C.L. and Driscoll, E.J. Methahexitol in dental surgery. Anesthesia and Analgesia, Current Research. Nov.-Dec. 1960.

Serial No. NIDR-56 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Serial Cephalometric Study of Postural Changes in Maxillary and Mandibular Full Dentures.

Principal Investigator: Dr. P. J. Coccaro

Other Investigators: Dr. R. S. Lloyd

Cooperating Units: Dental Department of the Clinical Center, NIH.

Man Years:
Total: 1/3
Professional: 1/3
Other: None

Project Description:

Objectives:

A serial cephalometric analysis of lateral roentgenograms to determine changes in a superior-inferior and anterior-posterior direction of the maxillary and mandibular full denture.

Methods Employed:

Tracings were made of all lateral head X-rays taken with the teeth in centric occlusion. Skeletal anatomical landmarks and reference planes were used to establish stable boundaries which would permit linear measurements from them to points on the maxillary and mandibular central incisors and thus demonstrate postural changes of the dentures on a time basis.

Patient Material:

Twenty-two patients, sixteen males and six females, with an age range of 25 to 62 years were admitted to the Clinical Center for the purpose of constructing full maxillary and mandibular dentures. Cephalometric lateral roentgenograms were taken on each of the patients at the following periods: (1) pre-surgery, (2) insertion of dentures, (3) 6 months post insertion, and (4) 12 months post insertion. Patients were then divided into 3 groups: (1) those showing an increase in the occlusal vertical dimension, (2) those showing a decrease in the occlusal vertical dimension, and

Part A (continued)

(3) those showing no change in the occlusal vertical dimension.

Major Findings:

1. The mandibular denture in all groups reflected a backward and downward position when analyzed at the time of insertion.

2. The period between denture insertion and 6 months after insertion showed the mandibular denture assuming a more posterior and inferior position. A similar change appeared during the 6 to 12 months post denture insertion period. All groups, however, showed the greatest directional change during the first 6 months.

3. The maxillary denture, for all groups, tended to show a progressive upward positioning from the time of insertion to 12 months after insertion. However, the greatest change was apparent during the first 6 months of denture wear.

4. In the antero-posterior direction, the maxillary denture reflected a posterior position at the time of insertion for all groups. However, from insertion to 12 months post insertion, variations existed within each group. Thus, "closed" groups showed a tendency toward stabilization of the denture while "open" groups showed a more positive forward position of the denture. Within each group, however, there was a tendency to reflect a more posterior position with time.

5. Mandibular and maxillary denture analyses of all groups showed findings similar to those for individual groups.

Significance to Dental Research:

These studies contribute information that may be used to assist in the construction of more stable dentures. For example, it may be possible to correlate changes in the maxillary and mandibular denture areas and estimate the effect they will have on the occlusal vertical dimension.

Proposed Course of Study:

To continue the study period beyond the one year post denture insertion evaluation.

Part B included: No

Serial No. NIDR-57 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Serial Cephalometric Analysis of Facial Height with Natural Dentition and Following Full Denture Construction.

Principal Investigator: Dr. P. J. Coccaro

Other Investigators: Dr. R. S. Lloyd

Cooperating Units: Dental Department of the Clinical Center, NIH.

Man Years (calendar year 1960):
Total: 1/3
Professional: 1/3
Other: None

Project Description:

Objectives:

1. To analyze skeletal and soft tissue facial profiles with natural teeth in occlusion and subsequently with full dentures in occlusion at the time of their insertion. Then again at 6 and 12 months after their insertion.

2. To observe changes discernible in the occlusal vertical dimension at the time of insertion of full denture and for the 6 and 12 month periods following insertion.

3. To evaluate, on a time basis, morphologic facial height and correlations with alterations in the facial profile.

4. To determine the influence, on morphologic facial height, of increasing or decreasing the occlusal vertical dimension at the time of insertion of full dentures.

5. To evaluate such changes, if any, on a time basis and determine what portion of the facial height is involved and to what degree.

Methods Employed:

1. This study was made possible through cephalometric roentgenograms taken before patients have had all of their remaining teeth removed prior to the construction of full dentures.

Part A (continued)

The technique used for taking the lateral headplates is well known and has been described by Broadbent.

2. Twenty-two patients with an adequate number of teeth in the upper and lower jaws to act as occlusal stops essential for obtaining occlusal vertical dimension measurements were selected for this study. All patients were to have their remaining teeth removed for reasons of advanced destructive lesions.

3. For the present study, 4 lateral cephalometric roentgenograms were taken on each of the patients. The first was taken with remaining natural teeth in centric occlusion. With artificial teeth in centric occlusion lateral roentgenograms were repeated at the time of inserting the full dentures and subsequently at a 6 and 12 month period after the patient had worn his dentures. Centric occlusion was used in this study since the present investigation covers an appraisal of the morphologic face height which is determined by the occlusal position of the dentition in the upper and lower jaws.

Approximately 88 films were traced and analyzed. Patients were classified into three groups depending upon what change was noted between the initial occlusal vertical dimension measurement and the second one obtained at the time of inserting full dentures. On this basis it was possible to establish three groups: (1) No change in occlusal vertical dimension; (2) Increase in occlusal vertical dimension; and (3) Decrease in occlusal vertical dimension.

The cephalometric roentgenograms employed in this study were placed upon an illuminated tracing table, covered with transparent matted acetate paper, and the anatomical landmarks traced. Correctional scales cut from each film were used and linear measurements determined for the upper face, middle face, lower face and total face.

Major Findings:

1. Discernible changes in the upper face appear to be minimal. Statistically, there appears to be no significant differences among "closed", "open" or "same bite" groups.

2. The morphologic facial height difference found between the pre-surgery and the denture insertion periods for groups whose "bite" was opened or closed was determined to be statistically significant.

Part A (continued)

3. Changes in morphologic facial height from insertion to 6 months proved to be greater than from 6 to 12 months following the insertion of full dentures. The collective data for all groups reflected more changes in the anterior and mandibular regions of the middle face. This appeared to be true for the period between denture insertion and 12 months following denture insertion as well as the period between insertion and 6 months post-denture insertion.

4. The mandibular region of the middle face showed slightly more change by comparison with the maxillary region. This appeared to be true for either the anterior or posterior regions.

5. There was little or no change in lower facial height during the periods studied.

6. The data for all groups studied showed a slight change in total facial height at insertion time and progressive reductions from the time of insertion to 12 months post-insertion. The greatest change occurred following the first 6 months of denture wear.

7. Most of the change noted in the morphologic facial height appeared to be within the middle facial region of the total face for all intervals studied.

Significance to Dental Research:

1. The present study will more scientifically document changes in morphologic facial height when patients have at time of full denture insertion: (1) An increase in occlusal vertical dimension, (2) A decrease in occlusal vertical dimension, and (3) No change in occlusal vertical dimension.

2. The study further demonstrates the important part cephalometric roentgenography can play in achieving more satisfactory and functional full denture construction.

3. The study makes possible the important collection of permanent pre-extraction records of skeletal and soft tissue facial profiles on patients long before they may even need full dentures. A clinical record of morphologic facial height in balance with facial musculature and the dentition would be invaluable to a prosthodontist at the time of full denture construction.

Proposed Course of Study:

This study is in continuing progress. Present cephalometric

Part A (continued)

roentgenograms are being taken for periods of 18 months and over on all patients in this study.

Part B included: No

Serial No. NIDR-58 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on Experimental Calculus Formation

Principal Investigator: Dr. P. N. Baer

Other Investigators: None

Cooperating Units: Dr. R.M. Stephan, Clinical Investigations Branch, NIDR; and Mr. Carl L. White, Epidemiology Branch, NIDR.

Man Years (calendar year 1960)
Total: 1/3
Professional: 1/3
Other: None

Project Description:

Objectives:

1. To investigate the effects of age, sex, strain, high carbohydrate diet, high protein diet on calculus formation in the rat.

Methods Employed:

Sprague-Dawley rats, 1-11 months of age, of both sexes were used. They were fed one of the following diets, high in carbohydrate (sucrose, glucose or cornstarch) or high in protein (casein). The experimental period varies from 30-70 days.

Major Findings:

1. Dental calculus can be produced in Sprague-Dawley weanling rats in 30 days on diets of a fine physical consistency and relatively high caloric content.

2. A carbohydrate diet containing 66% cornstarch produced more calculus than one containing either 66% sucrose or glucose.

3. A diet containing 62% protein and 13% sucrose did not produce more calculus than one containing 25% protein and 50% sucrose, all other ingredients being identical.

Part A (continued)

4. Older rats (6-11 months of age) do not develop dental calculus as readily as younger animals (1-3 months of age).

Significance to Dental Research:

Calculus is believed to be an important etiologic factor in periodontal disease; therefore, all knowledge which can be obtained concerning its formation should be of interest.

Proposed Course of Project:

Further studies on nutritional aspects of calculus formation will be pursued.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

1. Baer, Paul N., Stephan, Robert M., and White, Carl L.: Studies on Experimental Calculus Formation in the Rat: I. Effect of Age, Sex, Strain, High Carbohydrate, High Protein Diets. Journal of Periodontology (in press).

Serial No. NIDR-59 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies on Periodontal Disease in the Mouse

Principal Investigator: Dr. P.N. Baer

Other Investigators: None

Cooperating Units: Epidemiology Branch, NIDR; Biometrics Branch, Division of Research Services; National Cancer Institute.

Man Years (calendar year 1960):
Total: 1/3
Professional: 1/3
Other: None

Project Description:

Objectives:

1. To study the effect of a high protein, low carbohydrate diet on periodontal disease.

2. To investigate the role of maternal and genetic factors on periodontal disease.

Methods Employed:

1. Inbred STR/N mice were fed one of the following diets for an additional 90 day period after being maintained on Purina Laboratory Chow for a 170 day period:
   (a) A high protein (casein 62%) low carbohydrate (sucrose 13%) diet.
   (b) A normal protein (casein 25%) carbohydrate (sucrose 50%) diet.
   (c) A Purina Laboratory Chow diet.

2. Strain STR/N, which is susceptible to periodontal disease, was mated with strain DBA/2JN, which is resistant to periodontal disease. A total of 217 first generation mice were produced and these were then bred and yielded 435 second generation mice. In addition, four backcross generations were also completed, which yielded a total of 299 mice. Half the animals in each

Part A (continued)

generation were fed Purinal Laboratory Chow and the other half a high fat diet, (crisco 57%).

Major Findings:

1. A diet containing 62% protein in the form of casein, and 13% carbohydrate in the form of sucrose, when fed to STR/N mice, resulted in a statistically significant reducation in alveolar bone loss.

2. (a) The STR/N and DBA/2JN lines of mice evidently differ at a large number of loci, each of which has a small effect on periodontal disease.
   (b) The two strains differ by some maternal extra-chromosomal influence which may be passed on for at least two generations.
   (c) The extreme differences between the means of the two pure strains are due to both genetic and extra-chromosomal maternal factors.
   (d) There is considerable uncontrolled environmental variation in the expression of periodontal disease.
   (e) Mice fed the high fat diet show significantly less periodontal involvement than mice fed the stock diet.
   (f) Females whos slightly more periodontal involvement than males.

Significance to Dental Research:

These studies should contribute to our knowledge of the effect of diet and nutrition, as well as maternal influences, on periodontal disease.

Proposed Course of Project:

To continue the study of dietary and maternal influences on periodontal disease.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B; Honors, Awards, and Publications

Publications other than abstracts from this project.

1. Baer, Paul N. and White, Carl L.: Studies on periodontal disease in the mouse: IV. The effect of a high protein, low carbohydrate diet. Journal of Periodontology (in press).

Serial No. NIDR 60 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Histopathology of the Human Pulp

Principal Investigator: Dr. H. R. Stanley

Other Investigators: Dr. H. Swerdlow and Dr. R. Dubner

Cooperating Units: Dental Department, Clinical Center.

Man Years (calendar year 1960)

Total:	1
Professional:	½
Other:	½

Project Description:

Objectives: To determine:

1. The healing capacity of normal and diseased pulps following drilling procedures *per se*.

2. What characteristics of pulp pathology are the result of pressure changes when frictional heat is neutralized.

3. The possible additional irritating qualities of permanent and temporary filling materials compared to zinc oxide and eugenol.

4. The traumatic tendency of hand plugging versus pneumatic plugging of amalgam restorations.

5. The mitotic frequency of the odontoblasts and other less differentiated cells of the pulp.

6. The range of pathologic findings to be expected in the pulp resulting from various degrees of carious involvement.

7. The amount of pathology that might be produced by super-cooling of teeth by air-water spray techniques.

8. The characteristics of true pathologic alterations of pulpal tissue differentiated from those resulting from autolysis due to inadequate fixation; and to then establish the progressive pattern of autolytic disruption from the earliest, most

subtle, microscopic changes to complete destruction of the pulpal tissues.

9. The contribution of argyrophilic granules within odontoblasts to the formation of Korff's fibers.

10. The relative or true increase in pulpal collagen due to aging.

Methods Employed:

The principal method employed is the formalin fixation of extracted human teeth, followed by decalcification in 5% formic acid, paraffin embedding, serial sectioning, and staining with hematoxylin and eosin. Mason's trichrome, Wilder's reticulum stain, Feulgen's reaction, periodic acid-Schiff, toluidine blue, and other special stains are also utilized. The measurement of the remaining dentin thickness is obtained with a micrometer ocular. To establish autolytic changes, teeth are placed in formalin immediately following extraction, either intact or with their roots cut off at various levels up to the cervical line. Other teeth are permitted to bench-dry for various periods of time ranging from 15 minutes to 24 hours, followed by severance of the crown from the root and placement in formalin to permit rapid and maximum penetration of the fixative. These specimens are then decalcified and processed routinely.

Major Findings:

1. Speeds of 50,000 rpm and over, with instruments utilizing the belt-driven or turbine principle, have been found to be less traumatic to the human pulp than techniques utilizing 6,000 and 20,000 rpm with the conventional, ball-bearing handpiece. The value of coolants became more significant at the higher speeds. In the absence of adequate coolants, intermittent grinding was of no appreciable benefit. Preparation time at the higher speeds was of little consequence provided frictional heat was controlled. The combination of high speed, controlled temperature, and light load was conducive to minimal pulpal pathologic alteration.

2. Odontoblasts have been considered incapable of mitotic division. Preodontoblastic cells in the cell rich layer are, however, believed capable of dividing into cells which differentiate into odontoblasts as needed. With the accumulation of a number of specimens revealing mitotic divisions approximating predentin, this phase of pulpal regeneration must be re-evaluated.

3. Some carious teeth present little or no pulpal pathology, whereas, others present severe changes. A survey of extracted carious teeth needs to be undertaken to determine what depth of carious penetration must be reached and what other factors are required before pathologic alterations appear in the pulp.

4. Coolants, although adequate to prevent burn lesions, did not minimize the inflammatory responses when the operative technique required an applied force above eight ounces. Pressure of itself can induce a pulpal inflammatory response.

5. Clinicians report that patients complain of pain from the coldness resulting from the air-water sprays accompanying the use of the air turbines. Conventional speeds only utilize about 25 cc/min. as compared to the turbines using 35-75 cc/min. It has been shown that water temperature drops 15-20^{0}F. when converted to an air-water spray. If body temperature water is used, the expected drop in temperature when converted to a spray would still be confined to the desired range of +20^{0} F. ambient.

6. In the fixation study, many teeth that were bench-dried prior to fixation were better preserved than those placed without root amputation immediately in formalin following extraction. Larger teeth were more difficult to preserve than smaller teeth. Older teeth were more difficult to fix than younger teeth because of the lengthening of the roots by hypercementosis which increased the distance the formalin had to travel to reach the coronal pulp. Also, the diameter of the foramina, usually being smaller in older teeth, hinder the penetration of formalin. Good fixation of pulp tissue can seldom be obtained by placing extracted teeth *in toto* into fixative.

7. Many investigators have stressed that older teeth are more resistant to physical and chemical irritation than younger teeth. When young and old teeth, having been subjected to the same traumatic experiences, were compared, no obvious benefit from age was discerned.

8. Odontoblasts are not considered capable of producing von Korff's fibers. In view of the fact that these fibers are found between the cell membranes of odontoblasts, it is difficult to concieve of them being formed in the deeper tissues with eventual contouring of disoriented reticulin fibers into the characteristic spiral fibers of von Korff as the receding odontoblasts approach them.

Significance to Dental Research

1. Contradictory findings in the dental literature leave the profession in a quandary concerning the use of certain operative techniques and filling materials. Much of this confusion apparently has emanated from the lack of appreciation of various investigators for the response of the pulp to operative trauma *per se*. Pulpal response can vary in incidence and intensity according to the speed and pressure employed in instrumentation, the thickness of remaining dentin, the post-operative extraction period, the type of coolant, and the size of the cutting tool. All these factors must be allowed for before evaluating accurately the additional irritating properties of permanent and temporary filling materials, cavity liners, and sterilizing agents.

Information derived from such studies will be valuable to the entire dental profession.

2. Establishment of recognized characteristics of autolysis should lessen the difficulty of interpreting microscopic sections of pulpal tissues.

3. Occasionally, dental clinicians and investigators have advocated leaving behind some decay when a pulpal exposure appears likely. This procedure is based on the premise that calcium hydroxide, because of its caustic effect when placed into the cavity preparation on top of remaining caries, will inactivate the caries and stimulate the laying down of irregular dentin. However, such treatment can be dangerous since the function of Ca OH has not been proven.

Proposed Course of Project:

1. Since we have shown that high speed techniques are biologically safe for the human pulp, emphasis will now be placed on the added effects of filling materials.

2. Since forces above eight ounces have been shown to increase the intensity of the inflammatory response resulting from cavity preparation, evaluation of hand amalgam pluggers versus pneumatic instruments is in order.

3. The method for determining the amount of force employed in our operative studies, employing the Hamilton Postal Scale was coarse. New and more precise electronic equipment has been developed that permits continuous readings of the applied forces during the entire cutting procedure. Such instrumentation would provide a more direct and accurate recording of these measurements and will be used in further studies of this kind.

Part B included: Yes

Serial No. NIDR 60 (c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Stanley, H. R., Jr. and Swerdlow, H. Biological effects of various cutting methods in cavity preparation (The part pressure plays in the pulpal response). J.A.D.A. 61:450-456, Oct. 1960.

2. Stanley, H. R., Zander, Robinson and Bernier. Symposium on pulp reaction to operative procedures. J. of Oral Surg., Oral Med., and Oral Path. 13:329-330.

3. Stanley, H. R. and Swerdlow, H. The potential abuse of high speed technics. Dental Clinics of North America, Nov. 1960, page 621-627.

4. Stanley, H. R. Factors of age and tooth size in human pulpal reactions. Accepted for publication in J. of Oral Surg., Oral Med., and Oral Path. (In Press)

Awarded Honorary Professorship of the Dental School of the University of San Carlos, Guatemala City, Sept. 8, 1960.

Serial No. NIDR-61 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Temporomandibular Joint and its Relationship to the Masticatory Musculature and the Occlusion of the Teeth

Principal Investigator: Dr. R. Dubner

Other Investigators: Dr. P.N. Baer, Dr. P.J. Coccaro, Dr. A.D. Merritt

Cooperating Units: Electromyography Section, National Institute of Neurological Disease and Blindness; Diagnostic X-ray Department, Clinical Center; Normal Volunteer Group, Clinical Center.

Man Years (calendar year 1960):
Total: ¼
Professional: ¼
Other: None

Project Description:

Objectives:

1. To determine what role muscular forces and occlusal disharmonies play in temporomandibular joint disorders.

2. Evaluation of possible differences in the masticatory musculature and temporomandibular joint configuration of "normal control" patients and those in the disorder group.

3. Evaluation of various treatment procedures in cases of temporomandibular joint dysfunction.

Methods Employed:

Through the study of "normal controls" and patients with temporomandibular joint disorders an attempt is made to learn about the etiologic factors involved in the temporomandibular joint syndrome. Emphasis is placed on history, physical findings and various diagnostic methods, though therapy is necessarily an important phase of the procedures.

Diagnostic procedures include:
1. Complete medical history, examination and laboratory workup (blood and urine tests, chest X-ray, EKG, etc.)

Part A (continued)

2. Complete history and clinical examination of the temporomandibular joint illness.

3. Radiographic examination including temporomandibular joint laminographs and lateral plate cephalometrics.

4. Electromyographic studies of the muscles of mastication.

5. Psychiatric consultation when necessary.

Therapeutic procedures include:

1. Double-blind study of muscle relaxant drugs.

2. Occlusal therapy consisting of removal of centric and balancing side disharmonies.

3. Therapeutic exercises of the jaw.

Patient Material:

Patients included in the study are those with complaints of pain in the temporomandibular joint or masticatory musculature areas associated with clicking, subluxation and/or limitation in mandibular movement. Organic disorders such as osteoarthritis, rheumatoid arthritis, fractures and neoplasms are ruled out during the complete medical workup.

Normal volunteer patients at the Clinical Center who do not manifest any disturbance of the masticatory system are also given the same diagnostic workup as the disorder group.

Fifty patients in each group will be studied.

Major Findings:

At the present only ten patients with temporomandibular joint disorders and thirty normal volunteer patients have been studied. No conclusions have been reached at this early date in the study.

Significance to Dental Research:

Disorders related to the temporomandibular joint area have been a concern of modern dentistry for over forty years. Disharmonies of the occlusion of the teeth have previously been implicated as a major cause of these disorders. Since 1950, the trend has been towards emphasis on the role of mandibular muscle dysfunction in these cases. Greater understanding of the physiology of the interrelationship of these structures will aid in determining the etiologic factors involved.

Part A (continued)

Proposed Course of Project:

The proposed number of patients in both groups will be studied and evaluated. The knowledge gained will aid in directing us to new investigative approaches of this important problem.

Part B included: No

Serial No. NIDR-62 (C)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Studies in Human Saliva

Principal Investigators: Dr. R. O. Wolf and Dr. A. D. Merritt

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):

Total:	1
Professional:	3/4
Other:	1/4

Project Description:

Objectives:

1. To define the protein constituents of saliva.

2. To determine possible genetic markers in the salivary protein products of man and to correlate them with the serum protein products.

Methods Employed:

Saliva is analyzed by means of paper, agar, and starch electrophoresis. The agar electrophoresis has been combined with subsequent immunologic procedures (immunoelectrophoresis). The antibodies for the latter procedure have been prepared by the injection of appropriate sera and saliva into rabbits. Saliva is collected by means of a modified Lashley cup.

Patient Material:

Patients have been collected from those ordinarily seen in the Dental Outpatient Department.

Major Findings:

It is possible to separate the protein components of saliva into as many as six compartments. Most of these proteins are highly

negatively charged and migrate much differently than the serum proteins of the same patient. Distinct and reproducible immunologic reactions are obtained which do not tend to cross-react with serum antiserum similarly produced. In addition, saliva antiserum does not react with serum from the same patient.

Significance to Dental Research:

The salivary proteins have been studied meagerly in the past and it is thought that a further definition of these components will be of significance to the broad field of dental research. In addition, the possible polymorphic salivary protein pattern may prove to be another genetic marker in man.

Proposed Course of Project:

To extend and further define the salivary protein components of human saliva.

Part B included No

Serial No. NIDR 63 (c)
1. Clinical Investigations
2. Medical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Genetics Studies in a Population Isolate (Brandywine) with Particular Reference to Hemoglobin and Haptoglobin Patterns.

Principal Investigators: Dr. A. D. Merritt and Dr. C. J. Witkop

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960):

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description:

Objectives:

1. To utilize the haptoglobin and hemoglobin patterns within the population isolate as markers for the study of disease processes and other biologic data available from these patients.

2. To identify unusual hemoglobin and haptoglobin patterns and to define the types of haptoglobins produced when analyzing sibships in this population.

Methods Employed:

Sera and hemoglobin were collected from the Brandywine population isolate and studied by means of starch gel and paper electrophoresis respectively. The genetic types of haptoglobin and hemoglobin are being defined and will be analyzed by standard statistical methods.

Patient Material:

The patient source material for this study is derived from a major triracial isolate situated in the Brandywine, Maryland area. Most of the samples have been collected in field studies.

Part A (Continued): Serial No. NIDR 63 (c)

Major Findings:

Sickle cell hemoglobin is found in approximately 20% of the population (national average approximately 10% in a Negro population) and 2-1 "modified" haptoglobin patterns have been found and in a correspondingly increased percentage. The progeny from 2-1 "modified" haptoglobin parents are being defined.

Significance to Dental Research:

These genetic markers will serve for a correlation point for other disease states, and abnormal and normal findings within this triracial isolate. The general significance lies in the further definition of the pattern by which man inherits his normal and abnormal characteristics and the possible influence of genetically determined traits, one upon the other.

Proposed Course of Study:

These data are being analyzed and prepared for publication.

Part B included: No

Serial No. NIDR-64 (c)
1. Clinical Investigations
2. Medical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Oral Glucose Tolerance Test in Patients With and Without Periodontal Disease.

Principal Investigators: Dr. A. D. Merritt and Dr. P. N. Baer

Cooperating Units: None

Man Years (calendar year 1960):
Total: 1/3
Professional: 1/3
Other: None

Project Description:

Objectives:

1. To investigate the oral glucose tolerance test in patients with periodontal disease.

2. To attempt to clarify the relationship between glucose tolerance and periodontal disease.

Methods Employed:

Patients referred to the Dental Clinic and selected for periodontal disease therapy are fed a diet consisting of 300 grams of carbohydrate for no less than 3 days following which a standard oral glucose tolerance test is performed with the collection of specimens extending to the sixth hour. A complete medical and indicated laboratory investigation is also performed. A family history with particular attention to the possibility of diabetes mellitus is obtained.

Major Findings:

In the first 40 patients with advanced periodontal disease so far studied, 25% were found to have a decreased tolerance to the oral glucose load and 2 patients were found to have diabetes mellitus in need of therapy. It is considered that these ten patients all represent a probable pre-diabetic group.

Part A (continued)

Significance to Dental Research:

A possible association between glucose tolerance and periodontal disease is suggested.

Proposed Course of Study:

It is proposed to extend and analyze the data from an enlarging group of patients with periodontal disease and patients with known hyper and hypoglycemia.

Part B included: No

Serial No. NIDR-65 (c)
1. Clinical Investigations
2. Medical Investigations
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Studies of the Urinary Excretion Products of Histidine in Patients with Liver Disease.

Principal Investigator: Dr. A. D. Merritt

Other Investigators: Drs. M. Silverman, NIAMD and R. C. Gardiner, NIAMD

Cooperating Units: NIAMD

Man Years (calendar year 1960):

Total: 1

Professional: 3/4

Other: 1/4

Project Description:

Objectives:

1. To test the sensitivity of histidine loading as an indication of liver dysfunction.

2. To determine the effects on this test of cyanocobalamine, folic acid, and methionine.

Methods Employed:

Patients with liver disease of variable etiology are fed a standard dose of histidine. Urine, prior to and following histidine, is analyzed by a microbiological assay for metabolic products of histidine. A complete history and physical examination, blood counts, urinalysis, and multiple liver function tests are obtained on all patients.

Major Findings:

1. Formiminoglutamic acid (FGA) and urocanic acid have been identified in patients with liver disease, and unusual

Part A (continued)

amounts of these substances are found following oral loading with histidine. Normal patients do not exhibit these findings. The products (FGA and urocanic acid) have been isolated and identified by their melting points and ultra-violet and intra-red spectroscopy. The sensitivity of this functional capacity of the liver as an indication of liver disease was investigated and compared with the usual liver function tests in 28 patients.

2. No effects have been found following the administration of cyanocobalamine and methionine. Folic acid partially corrects the defect but not to the degree expected in malnutrition (malabsorption syndrome, folic acid deficiency).

3. Under particular circumstances the urinary excretion of urocanic acids excedes that of FGA (2 of 28 patients). The reason for this is unknown.

Significance to Dental Research:

Liver dysfunction is measurable with this test. Histidine metabolism, in man, proceeds via trans-urocanic acid as a major pathway. This has contributed to our knowledge of metabolic processes and has been related to other studies in NIDR including, for example, metabolic processes of saliva.

Proposed Course of the Project:

Completed during 1960.

Part B included: No

Serial No. NIDR-66 (C)
1. Clinical Investigations
2. Medical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Renal Function in Patients with Sjögrens Syndrome.

Principal Investigators: Dr. A. D. Merritt and Dr. M. Kahn (NHI)

Other Investigators: None

Cooperating Units: Laboratory of Renal Physiology, National Heart Institute

Man Years (calendar year 1960):

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description:

Objectives:

To identify and characterize a renal defect in urine concentration present in patients with Sjögrens Syndrome.

Methods Employed:

The ordinary clinical chemistry tests, history and physical examination were used to define Sjögrens Syndrome. Standard water deprivation and water loading tests were performed with and without estimations of the solute and inulin clearances.

Major Findings:

It has been found that some patients with Sjögrens Syndrome have a variable inability to concentrate the urine which is not responsive to increased amounts of antidiuretic hormone. The renal clearance of inulin is only slightly decreased and as there is an apparent ability to conserve sodium, glucose, and other electrolytes as well as an ability to excrete urea, the total renal tissue is felt to be quantitatively adequate. Evidence for vasculitis obtained by skin biopsy as well as the abnormally functioning salivary glands coupled with a normal ability to dilute the urine and a decreased ability to concentrate the urine suggests a defect in the distal convuluted tubules or in the collecting ducts of the kidney.

Part A Cont'd. Serial No. NIDR-66 (C)

Significance to Dental Research:

The abnormal physiologic findings in patients with Sjögrens Syndrome have been fully extended and some insight obtained into the diffuse derangements associated with this disorder.

Proposed Course of Project:

Studies completed during 1960.

Part B included No

Serial No. NIDR-67 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Investigations into the Etiology of Recurrent Aphthous Ulcerations and Other Oral Nonmalignant Soft Tissue Diseases.

Principal Investigator: Dr. I. I. Ship

Other Investigators: None

Cooperating Units: University of Pennsylvania, Dental School

Man Years:

Total:	1 1/2
Professional:	1/2
Other:	1

Project Description:

Objectives:

1. To study and isolate the various external and environmental etiological factors involved in the pathogenesis of recurrent aphthous ulcerations and other nonmalignant, chronic, and recurrent oral soft tissue diseases.

2. To determine the experience and prevalence of recurrent aphthous ulcerations as well as related systemic conditions in a graduate school population.

3. To investigate and elucidate the gross and microscopic pathology of the lesions.

4. To evaluate various new agents for the control and prevention of recurrences of these conditions.

5. To investigate and clarify the multiple diagnostic criteria for this group of diseases.

6. To investigate the relationship of adrenal-cortical activity to the formation of aphthous ulcerations and other recurrent oral diseases.

Part A (continued)

1. Examination includes:

   a. Viral cultures and serological antibody testing.

   b. Allergic tests with the utilization of synthetic diets on hospitalized patients.

   c. Hemotological studies

   d. Eosinophil counts and plasma cortical steroid determination.

   e. In vivo microscopy and microphotography.

   f. Biopsy and pathological examination.

   g. Clinical consultation.

2. a. Administration of questionnaire to 1800 graduate school stuents at the University of Pennsylvania to determine the incidence and experience of recurrent aphthous ulcerations and recurrent herpes labialis as well as other oral soft tissue diseases.

   b. Active follow-up of this group with examination of past medical history, review of systems, and clinical oral examinations.

   c. The maintenance and review of monthly diary forms recording duration, location, and severity of individual lesions in each patient for a period of 18 months.

3. Experimentation with new forms of topical anesthetic agents with low toxicity and high potency for supportive therapy.

4. Double blind clinical investigations of agents for prophylactic administration to prevent recurrences of aphthous ulcerations. Special diary forms recording location, duration, and severity as well as number of individual lesions during test periods on specific double blind medications provide the criteria for the evaluation of all agents.

Patient Material:

1. Individuals suffering acute, active disease referred from the local community and maintained as outpatients on experimental therapeutic regimes, or admitted as in-patients to the Clinical Center for careful follow-up.

Part A (continued)

2. Students attending the University of Pennsylvania Schools of Dentistry, Medicine, Veterinary Medicine, and Graduate Nursing.

3. Student nurses in the University of Pennsylvania Nursing School, the Presbyterian Hospital Nursing School, and the Philadelphia General Hospital School of Nursing.

4. Students of the Dental Hygientist School of the University of Pennsylvania

Major Findings:

1. Tissue culture technics (using monkey kidney, human skin, rabbit kidney, and hela cells) have failed to isolate herpes simplex virus or other viruses from 100 aphthous ulcerations (in various stages of development) in 31 patients. Herpes simplex virus was, however, isolated from 13 of 25 recurrent herpes labialis vesicles in 11 patients.

2. Acute exacerbations of aphthous ulcerations with the formation of multiple acute lesions appear to coincide with periods of emotional stress in many patients. No cause and effect relationship has been demonstrated.

3. In contrast to previous reports in the literature, recurrences in females in the present study did not follow a menstrual cycle. Remission during pregnancy, however, has been demonstrated in six instances.

4. There was a failure of regression of lesions or remission of disease when patients were placed on synthetic, non-allergic diets with the elimination of all cosmetics, aromatic oils, and synthetic clothing. Dietary allergy did not have etiological significance in the progression of disease in the 8 patients examined in this manner.

5. Allergic etiology was demonstrated in one case.

6. Hydrocortisone, taken systemically or applied locally to the affected tissues, was beneficial in the prevention of recurrences and the regression of aphthous ulcerations. Analysis of initial results shows a dose-response relationship in most patients, (prophylactic efficiency being related to the level of drugs applied to tissues twice daily).

Part A. (continued)

7. A solution containing 0.1% cyclomen hydrochloride and 0.5% diphenhydramine hydrochloride applied topically to acute aphthous ulcerations gave significant temporary relief from pain, but had no effect on the severity and duration of individual lesions. This anesthetic solution was also demonstrated to have broad clinical effectiveness in many forms of painful stomatitis, including pemphigus vulgaris, leukemia, oral lichen planus, and stomatitis secondary to cancer chemotherapy.

8. 52.5% of males and 17.7% of females in the study reported positive history of recurrent aphthous ulcerations or canker sores. Differances between the sexes were significant (P=.02). However, no differences were noted as regards geographic origin, racial background, age, marital status, school or class. Significant religious group differences were noted.

9. 38.4% males and 38.0% females in this population had a history of recurrent herpes labialis or "fever blisters." Geographic origin, racial background, age, sex, marital status, school, and class showed no significant group differences. Significant religious group differences were once again noted, but these varied from the group differences seen in the aphthous ulceration category.

10. The interrelationships between both diseases were examined in the same group. Comparison of the expected and actual proportion of students with both diseases showed a relationship in females but not in males. Students with recurrent aphthous ulcerations experienced more frequent episodes of disease than students with recurrent herpes labialis. This fact was clearly demonstrated in examinations of the group that had both diseases.

Significance to Dental Research:

Investigations into the many individual and group factors in these diseases have led to increased information regarding their interrelationships in a population group, and could contribute to a better understanding of etiology and eventually to a rational method of treatment.

Proposed Course of the Project:

Termination of investigation as of July 1, 1960.

Part B included: Yes

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Ship, Irwin I., D.M.D., Williams, Anderson F., D.M.D., and Osheroff, Boris J., B.S.: The Development and Clinical Investigations of a New Oral Topical Anesthetic (Dyclonine Hydrochloride-Diphenhydramine Hydrochloride) for Acute and Chronic Oral Lesions. J. of Oral Surgery, Oral Medicine, and Oral Pathology, 13:630-636, May 1960.

2. Driscoll, E.J., D.D.S., Ship, I.I., D.M.S., Baron, S., M.D., Stanley, H.R., D.D.S., and Utz, J.P., M.D.: Clinical Aphthous Stomatitis, Herpes Labialis and Related Conditions. Combined Clinical Staff Meeting of the National Institutes of Health. Annals of Internal Medicine, 30:1475-1493, June 1959.

3. Ship, I.I., D.M.D.: The Diagnosis and Treatment of Ulcerations in the Mouth. Dental Health Memorandum, U.S.P.H.S., December 1, 1959.

4. Ship, I.I., D.M.D., Morris, A.W., D.D.S., Durocher, R.L., D.D.S., and Burket, L.W., D.D.S.: Recurrent Aphthous Ulcerations and Recurrent Herpes Labialis. I. Experience in a Graduate and Nursing School Population. J. of Oral Surg., Oral Med., and Oral Path. 13:1191-1202, 1960.

5. Ship, I.I., D.M.D., Morris, A.W., D.D.S., Durocher, R.L., D.D.S., and Burket, L.W., D.D.S.: Recurrent Aphthous Ulcerations and Recurrent Herpes Labialis in a Professional School Student Population. II. Medical History. J. of Oral Surg., Oral Med., and Oral Path. 13:131701329, 1960.

6. Ship, I.I., D.M.D., Morris, A.W., D.D.S., Durocher, R.L., D.D.S., and Burket, L.W., D.D.S.: Recurrent Aphthous Ulcerations and Recurrent Herpes Labialis in a Professional School Student Population. III. Oral Examinations. J. of Oral Surg., Oral Med., and Oral Path. 13: 1438-1444, Dec., 1960.

7. Ship, I.I., D.M.D., Morris, A.W., D.D.S., Durocher, R.L., D.D.S., and Burket, L.W., D.D.S.: Recurrent Aphthous Ulcerations and Recurrent Herpes Labialis in a Professional School Student Population. IV. Twelve Months Study of Natural Disease Patterns. J. of Oral Surg., Oral Med., and Oral Path. 14:30-39, Jan. 1961.

Serial No. NIDR-68 (c)
1. Clinical Investigations
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Stress in the Dental Patient

Principal Investigator: Dr. Irwin I. Ship

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1960)
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives:

1. To determine and compare changes in adrenocortical activity caused by various aspects of dental therapy in healthy adult humans.

2. To investigate the changes in adrenocortical responses to dental stress induced by the use of local anesthetics and simple barbiturate premedication.

3. To investigate the systolic and diastolic blood pressure responses to various aspects of dental therapy in healthy adult humans.

4. To establish a reliable index of physiological response for future testing in dental techniques and therapeutics.

Methods Employed:

1. All dental treatments are standarized and divided into six categories, interview, radiographic examination, oral examinations, prophylaxis, operative dentistry, and oral surgery.

2. Total eosinophil counts taken from capillary blood by standard finger puncture prior to dental therapy are compared with similar counts taken 4 hours following treatment.

3. Examination of minute-to-minute systolic and diastolic blood pressure changes during 40 to 60 minute dental appointments using the Colson Model 110 blood pressure recorder.

4. A 50 milligram pentobarbital sodium capsule, an identical placebo capsule, or no capsule is dispensed by the Pharmacy according to a regimen determined by a statistician and kept blind both from patient and investigator. Capsules are taken ½ hour prior to dental appointments.

5. Two cc of 2% Lidocaine solution, 2 cc of sterile saline, or no injection is made at each appointment according to a schema established by the statistician.

6. Controls for this study consist of a) ordinary dental appointments randomly selected as regards sequence of treatments at which time no procedures are attempted and patient rests for the entire time in a dental chair, b) four eosinophil counts taken at unscheduled times so as to indicate normal daily variations.

Patient Material:

Approximately 25 healthy adult employees of the National Institutes of Health with extensive dental caries referred by the Employee Health Service.

Major Findings:

1. A comparison of stress responses in the various treatment groups with responses in the control appointment groups revealed significant changes in the former.

2. Stress responses to various operative dentistry procedures demonstrated no significant differences when compared with responses to interview, X-ray and oral examinations, or prophylaxis appointments.

3. Local anesthesia had no effect on the stress reactions in operative dentistry.

4. Barbiturate premedication was associated with slight reduction in the stress reaction in operative dentistry while placebo had no effect.

5. The stress reactions to oral surgical procedures were greater than that to all other procedures tested. A substantial reduction in stress response in this group was established by barbiturate premedication.

6. Anxiety, fear, and apprehension during the course of the dental appointment appeared to be the primary factor involved in the stress inducing aspects of dentistry.

7. The effects of dental procedures and control of the blood pressure were investigated in 17 healthy adult subjects. Analysis of blood pressure results revealed the following:

a) Fluctuations in the range of 25 mm of mercury were observed around the mean systolic and diastolic pressures. These fluctuations were independent of age, sex, time of day, day of week, and initial blood pressure.

b) The various dental procedures showed insignificant net differences in either mean systolic or mean diastolic blood pressures.

c) Patients appeared to fall into three distinct categories of response to dental procedures and were classified as "nonreactors", "reactors", and "hyperreactors."

Significance to Dental Research:

Although little is known regarding physiological response to actual dental procedures in human beings, the subject is frequently discussed particularly in reference to newly developed dental instruments and techniques. A physiological tool for the evaluation of techniques and therapeutics in the dental field would be a valuable contribution to the profession.

Proposed Course of Project:

Termination of Study as of July 1, 1960

Part B included: Yes

Serial No. NIDR-68 (c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Ship, I.I., D.M.D., and White, C.L., A.B., Physiological Response to Dental Stress. Accepted for publication in the Journal of Oral Surgery, Oral Medicine, and Oral Pathology, 13:368-376, 1960 (March)

2. Ship, I.I., D.M.D., The Response of Systolic and Diastolic Blood Pressures to Dental Stress. Accepted for publication in the Journal of Oral Surgery, Oral Medicine, and Oral Pathology, 13: 449-507, 1960 (April)

Serial No. NIDR-69 (c)
1. Clinical Investigations
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Secretory Sialography in Patients with Sjögren's Syndrome and Rheumatoid Arthritis

Principal Investigator: Dr. I. I. Ship

Other Investigators: Dr. K. Block, NIAMD

Cooperating Units: National Institute of Arthritis and Metabolic Diseases

Man Years (Calendar year 1960):

Total: 1/4

Professional: 1/4

Other None

Project Description

Objectives:

To investigate salivary gland physiology and pathology in Sjögren's Syndrome in relation to Rheumatoid arthritis and other connective tissue diseases, and to study the oral structures in these diseases. To document the clinical, serological, immunological, and pathological spectrum of Sjögren's syndrome and related connective tissue diseases.

Methods Employed:

Sjogren's syndrome refers to a complex consisting of keratoconjunctivitis sicca, xerostomia and/or salivary gland enlargement and rheumatoid arthritis or one of the related connective tissue diseases. Dryness of the mucous membranes of the nose, pharynx, and bronchi, esophagus and stomach as well as that of the vagina may occur. Histological examination of the glands responsible for keeping these tissues moist reveals atrophy of acinar paranchyma, intensive lymphocytic infiltration and in the lacrimal and salivary gland, epi-myoepithelial islands.

Part A (continued)

Techniques for the physiological and pathological evaluation of major salivary glands have been developed and standardized. Secretory sialography is being done on each patient to define qualitative and quantitative gland changes associated with diminished secretory efficiency. Standard saliva collections using the Lashley Cup apparatus as well as gland biopsy followed by pathological evaluation contribute to clinico-pathological correlation, and further extension of our knowledge of these structures.

Clinical Aspects:

Twenty-one female patients with clinical evidence of Sjögren's syndrome were studied until the present time. The diagnosis of Sjogren's syndrome was based on symptoms related to dryness of mucous membranes and symptoms of the related connective tissue disease. Confirmatory examinations and laboratory studies were performed by cooperating units in the Ophthalmology Service (Dr. Richard Oglesby) and NIDR. The diagnosis of keratoconjunctivitis sicca was based on filamentary keratitis demonstrated by biomicroscopy, decreased tear flow demonstrated by Schirmer Test #1, and abnormal staining of the bulbar conjunctiva and cornea by Bengal-rose stain. Dental examination disclosed dryness of the oral mucous membranes, unusual pattern of dental caries in some patients and enlargement of major salivary glands. Parotid flow was measured by use of the Lashley cup and indicated marked reduction or complete absence of salivary flow in several patients in this group. (Dr. Robert M. Stephan, NIDR). On secretory sialography of the parotid gland sialectasis was demonstrated in all of the patients examined. (Dr. Irwin I. Ship, NIDR). Approximately 40 other patients have been examined by these techniques and serve as a control group. According to the American Rheumatism Association criteria for the diagnosis of rheumatoid arthritis the initial group of 21 patients was divided into four groups. In the first group were 8 patients with definite or classical rheumatoid arthritis; in the second group were three patients with possible rheumatoid arthritis; two had scleroderma and 8 patients had only oral and ocular manifestations of Sjögren's syndrome.

Laboratory Examinations:

An unusual finding in this group of patients was a combination of low fixed urinary specific gravity and low blood urea nitrogen. This may be related to the chronic

Part A (continued)

intake of large amounts of water. (This phase of our study is being conducted by Dr. Sheldon M. Kahn-NHI). Six of these patients had low white blood cell counts and many had eosinophilia. Two patients had thrombocytopenia without purpura. The erythrocyte sedimentation rate was elevated in 20 of 21 patients.

Serum Protein Changes:

Total serum proteins were less than normal in the first three groups of patients and were increased in the patients in group four. Concentration of serum albumin was below normal in the entire group. Serum globulin concentration was increased in all four groups; a marked increase was noted in the gamma globulin concentration, especially in the last group.

Serological Reactions:

The bentonite flocculation test (BFT) was positive in 20 of 21 patients and the sensitized sheep cell agglutination test was positive in 18 of 20 tested. The serum component responsible for the positive BFT was located in the bottom fraction or pellet obtained by ultracentrifugation through a sucrose density gradient. Sera from the four groups behaved similarly. The serologically active fractions contained less than 10% of the total protein used in each experiment and contained all of the high molecular weight gamma globulin (19S) as determined immunologically by gel diffusion using as antiserum to 19S gamma globulin.

Analytical ultracentrifugation (Dr. William R. Carroll) revealed unusual high molecular weight complexes with a sedimentation constant of approximately 22, only in sera from patients with Sjögren's syndrome and rheumatoid arthritis. (These 22S complexes have been described in approximately 30% of rheumatoid arthritis patients, especially those with very high titers in serological tests for rheumatoid arthritis.) Furthermore, these ultracentrifugation studies indicated that the increase in gamma globulin concentration was due chiefly to an increase in the 7S class of proteins.

Serum constituents with special affinity for tissue nuclei were demonstrated using fluorescent antihuman gamma globulin (Dr. Richard A. Malmgren). Significant titers were obtained with 13 of 21 sera from patients

Part A (continued)

in this study. In comparison, only 4 of 27 rheumatoid patients without Sjögren's syndrome had significant titers in this technique. However, only two patients had positive L.E. cell preparations; in one of these patients this was associated with clinical evidence of systemic lupus erythematosus.

Five of the patients in this group had positive direct Coomb's tests. Studies are proceeding to identify the antibody coating red blood cells in these patients. Three of the patients had antibodies to thyroglobulin coated, tannic acid treated sheep erythrocytes. There was no evidence of clinical thyroid disease in these patients.

Experimental Studies:

Attempts to demonstrate antibodies to saline extracts of human salivary gland have been inconclusive. Preliminary experiments using complement fixation indicated that sera of patients with Sjögren's syndrome reacted with saline extracts and fixed complement. These studies are to be further confirmed and extended.

Attempts to use saline extracts of submaxillary glands as antigen in agar gel diffusion studies also led to inconclusive results.

Experimental Production of Sjögren's Syndrome:

Rabbits were injected with homogenates of rabbit submaxillary glands emulsified in Freund's adjuvant. Rabbits were injected in the foot pads and in the intrascapular region.

These animals were observed for 5 months and no ocular or oral disease was observed. Repeated skin tests with homogenates of salivary gland were negative. Serial bleeding and testing did not reveal precipitating antibodies for salivary extracts. Histologic examination of tissue from two injected rabbits did not reveal significant abnormality in the salivary or lacrimal glands.

Similar experiments have been started in guinea pigs injected with homogenates of lacrimal glands emulsified in Freund's adjuvant. After five weeks these animals showed skin reactivity to homogenates of lacrimal gland. Histologic examination of tissue from these animals is pending.

Part A (continued)

Significance to Dental Research:

Sjögren's syndrome is a little known variant of rheumatoid arthritis. Preliminary studies suggest that it may be due to an autoimmune process which interferes with the function of glands responsible for moistening various mucous membranes. A better understanding of this syndrome may increase our knowledge of rheumatoid arthritis, related diseases, and salivary gland functions.

Proposed Course of Project:

1. Further attempts will be made to demonstrate antibodies to salivary and lacrimal gland constituents using complement fixation, precipitation, and agglutination techniques.

2. Additional animal experiments will be conducted in an attempt to reproduce Sjögren's syndrome.

3. Clinical studies will continue in order to define the full spectrum of this syndrome, including study of patients with only oral or ocular disease.

4. The renal defect in Sjögren's syndrome will be further defined.

5. Studies will continue to delineate the pattern of dental caries and abrasion seen in patients with Sjögren's syndrome.

6. Studies are planned to investigate the clinical and serological manifestations among the family members with this syndrome.

7. Investigations into the relationships of pathological, secretory, and sialographic changes in the salivary glands will be continued.

8. At present, two groups are being carefully examined in reference to salivary gland functions: patients with Sjögren's syndrome (with rheumatoid arthritis) and a group with rheumatoid arthritis without Sjögren's syndrome. The addition of a third, non rheumatoid arthritis, nor Sjögren's syndrome patients would provide an absolute control for the study.

Part B included: Yes

Serial No. NIDR-69 (c)

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Bloch, K.J., Wohl, M.J., Ship, I.I., Oglesby, R.B. and Bunim, J.J. Sjögren's Syndrome I. Serologic Reactions in Patients with Sjögren's Syndrome with and without Rheumatoid Arthritis. J. Am. Rheumatism Assoc., 3:287-297, 1960.

Serial No. NIDR-70 (c)
1. Clinical Investigations
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A.

Project Title: Effect of Dietary Phosphates on Dental Caries in Children.

Principal Investigators: Drs. I. I. Ship and O. Mickelsen, NIAMD

Other Investigators: Drs. F. J. McClure, R. C. Likins, I. Zipkin, A. L. Russell, and Mr. C. L. White, NIH
Dr. H. Schraer, The Pennsylvania State University
Drs. G. E. Waterman, B. Bosley, and Miss H. G. Olson, Division of Indian Health
Misses M. Talcott and B. Wenberg, South Dakota State College of Agriculture and Mechanic Arts

Cooperating Units: Division of Indian Health, DI
Home Economics Depart ment, South Dakota State College of Agriculture and Mechanic Arts
Department of Physics, The Pennsylvania State University
Bureau of Indian Affairs, PHS

Man Years (calendar year 1960):

Total: 1/3

Professional: 1/3

Other: None

Project Description:

Objectives:

To determine whether the addition of phosphates to the diets of children will reduce the incidence of dental caries; whether the addition of phosphates to the diets of children will effect the growth and development; and to evaluate the safety of the phosphates and calcium added to the diets.

Part A (continued)

Methods Employed:

Two percent calcium phosphate has been added to the bread in four boarding schools and the addition of a placebo (flour) to the bread in four control schools. Neither the subjects nor the investigators know the identity of the schools. Total number of children in the study is 1800. Routine examinations include an annual dental examination including bilateral bite-wing radiographs on all children; quarterly measurements of height and weight on all children; annual bone density determinations using hand radiographs on a sample of 200 children from both the control and supplemented schools; annual saliva collection and analysis on a sample of 200 children from both the control and supplemented schools; regular food intake surveys including collection and analysis of representative diets in all schools; annual physical exams for nutritional status on a sample of children in both control and supplemented schools; and annual blood and urine collections on a sample of children in both groups of schools for hemoglobin, calcium, phosphorus, and magnesium determinations.

Major Findings:

1. First year examinations showed no difference in either DMF rates or in appearance of new carious surfaces between any of the eight schools.

2. Analyses of saliva samples indicated no difference in the concentration of calcium, phosphorus, ash, and total solids between the children in the different schools; and there was no relationship between these salivary constituents and age or sex of the subjects.

3. The primary emphasis on the possible dangers associated with the supplemental calcium intake has related to possible kidney disturbances. After considerable consultation with kidney physiologists, it became apparent that there was no single simple test of kidney function. Perhaps the best measure of functional activities was the urine concentration test. An attempt was made to carry out a simplified version of a urine concentration test. At the evening meal, the children received only a minimum amount of fluid. From dinner-time until bedtime, an older child was placed at each drinking fountain to make certain that the children did not drink. The matrons instructed the children to urinate at bedtime and after that gave each child a plastic screw-capped bottle with the child's name on it. The instructions were that any urine passed during the night or upon arising in the morning was to be collected. The results from these preliminary tests were so equivocal, that it was felt inadvisable to extend them.

Part A (continued)

Significance to Dental Research:

1. Although it is well established that fluorine added to drinking water reduces the incidence of dental caries by approximately 65%, there is still a large increment of caries to be taken care of. Thus, a public health need exists to develop a means of reducing the incidence of dental caries among the people who do not have access to a fluoridated water supply. Phosphate supplementation in the diet may be a promising approach.

2. The addition of calcium to the diet offers an opportunity to evaluate the safety of long-term calcium supplements and its effects on growth and development. It also offers a means of securing an answer to a fundamental public problem; namely, will added calcium reduce the absorption and retention of strontium^{90}.

Proposed Course of the Project:

1. To evaluate the effectiveness of calcium supplementation in the diet as a means of reducing the absorption and retention of strontium^{90}.

2. To do controlled balance studies for Ca, P, Mg, and Sr^{90} on a small group of children from both the control and $CaHPO_4$ supplemented schools.

Part B included: No

Calendar Year 1960

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

OFFICE OF THE DIRECTOR

CC-01
Serial No.

The Clinical Center provides the specialized forms of hospital care necessary for patients participating in research programs of the categorical Institutes of NIH. Except for physician care, which is furnished by the Institutes, the Office of the Director is charged with all responsibilities for the admission, diagnosis, and treatment of patients. Care furnished to patients is of optimum quality. Each receives individual personal attention rendered with genuine warmth and interest by all persons concerned. In conducting its integrated laboratory-clinical research programs, the Clinical Center strives to attain the important objective of maintaining an environment in which productive medical research flourishes, and in which promising young physicians may have advanced training opportunities to fit them for careers in medical research. The total responsibility requires a continuing evaluation of all hospital departments and services.

The new Surgical Wing of the Clinical Center has now reached 74 per cent of scheduled completion. It will be dedicated towards the end of the current fiscal year. Long planned and painstakingly designed, this circular four story annex will provide unique facility for research in cardiac and neurological surgery. Embracing new concepts for construction of this type, its special adaption for utilization of television and photography for recording and transmittal purposes will greatly aid the teaching program.

Sources for the recruitment of Normal Volunteer control patients have been appreciably expanded. Twelve sponsoring agencies have current contracts with the Clinical Center. Most of those added this year will provide people of a much greater age spectrum than we have been able to obtain until now. This will enable us to expand the scope of our clinical research.

The Normal Volunteers constitute one of our most precious resources. Because the safety and well being of the individual are of paramount concern to the Clinical Center, they are surrounded by elaborate safeguards. In seeking approval for a research proposal, the investigator must first prepare a written description of the project in which he presents the purpose of the research; procedures to be followed; anticipated duration of the study; what will be required of the Volunteers; nature and extent of inconvenience and risk involved, and all related pertinent information. Clearances of the proposal are required from the Clinical Director of the Institute concerned; Clinical Research Committee of the Medical Board of the Clinical Center; Medical Board of the Clinical Center, and the Director, NIH.

Projects are approved only for the duration of the principal investigator's association with and direction of the specific project. Only qualified physicians or dentists who are NIH staff members may assume responsibility for clinical diagnosis, investigation and care. The principal investigator is personally responsible for providing the assigned Volunteers at the level of their individual comprehension, with sufficient information about the purpose, methods, demands, risks, inconveniences and discomforts of the study to enable each Volunteer to make a mature judgement as to his willingness and ability to participate. He is also responsible for obtaining the signed consent of each Volunteer to participate in each phase of the research project.

Admission and Occupancy Data

During 1960 the 516 beds of the Clinical Center were used to their planned percentage of capacity for the third successive calendar year. The 3,668 patients admitted during 1960 was an increase of 216 patients over 1959. The average daily census was 380, an average daily occupancy rate of 74%.

A total of 19,462 patients have been admitted to the Clinical Center since it started operation in 1953. Each was referred by his own physician or medical agency, and each was accepted because his case had already been diagnosed as having the particular kind or stage of a disease or other condition required for a current Institute research project. The number of patients admitted was approximately 10% of the physician referrals which had been formally reviewed and rejected as not meeting the criteria of any current study project.

Practicing physicians continued to make referrals at the same satisfactory level we had enjoyed during previous years. Information about the varying needs of the programs of clinical investigation was published in medical journals, professional "newspapers," and the lay press, as an aid and supplement to USPHS Publication No. 284, "Current Clinical Studies and Patient Referral Procedures." This publication was revised and expanded in July and distributed to a mailing list of nearly 6,000 interested physicians and health agencies.

National, regional and local contact with medical groups has given a better and firmer understanding of the mission and purpose of the Clinical Center in the study and treatment of the patients we accept.

Widespread sources of referral of patients was pursued as a matter of continuing policy. Of all patients admitted 50.22% came from Maryland, Virginia and the District of Columbia.

Summary

	1959	1960
Total number of admissions	3,452	3,668
Average daily patient census	384	380
Percentage of bed occupancy	74	74
Average hospital days per patient	41	37
Visitors to Admissions & Followup Dept.	25,070	26,387

Advanced Training of Physicians

As in any research environment, professional growth is an important component of the Clinical Center program. The young physicians chosen for the Clinical Associate and Residency training programs come to us from universities in which there are extensive research programs in the biological sciences. In all duty assignments given to them, there is a strong educational component consisting of work assignments within a research area. Professional growth develops through the process of intimate personal guidance by senior workers, coupled with selected educational experiences designed to advance both the work in progress and the competence of the young investigator.

This system has the advantage of providing the highest possible quality of care for patients while increasing the number of physicians trained in the methodology of clinical research. Residency training approved by an American medical specialty Board is offered in: Clinical Pathology, Pathologic Anatomy, Internal Medicine, Diagnostic Radiology, Psychiatry, Neurology, Ophthalmology, Dermatology, Oral Pathology and Periodentology. It is significant that the medical specialty boards approve all or part of the time served by junior members of the Clinical Center staff as credit training for specialty certification. The value placed on experience gained here is often a deciding factor in the recruitment and retention of staff.

Medical Board

The Medical Board of the National Institutes of Health serves a function and purpose parallel to that of the Senior Medical Committee of a university hospital. Composed of representatives from each of the Institutes and the Clinical Center, it establishes standards and recommends policies having to do with the care of patients participating in the research activities of the Clinical Center. Its committees are: (1) a Clinical Research Committee which must pass on all projects involving Normal Volunteers; (2) a Medical Record Committee which continually surveys and evaluates medical records kept on patients in the Clinical Center; (3) a Steering Committee which considers special problems pertaining to patient care; (4) a Credentials Committee which approves the qualifications of consulting physicians; (5) a Tissue Committee which studies the correlation of the pathologists' reports of tissues removed from surgery patients with the clinical diagnosis; (6) an Education Committee which advises on matters relating to post-graduate medical education conducted at the Clinical Center;

(7) a Normal Controls Committee which provides advice to the Medical Board on the recruitment, utilization, and other areas of interest involving "normal control" patients.

Meeting semi-monthly, the Medical Board concerns itself with such important matters as improved patient care, strengthening of the elan of the professional staff, and continuation of our harmonious relationship with referring physicians.

The Medical Board for 1960 had a new chairman and three other new members. There were a number of changes in appointments to the various committees of the Medical Board.

The Clinical Research Committee has been active again this year principally because of the number of projects involving normal volunteers.

The following four amendments were added to the Organization and By-Laws Document:

Amendment No. 14., Revision of first sentence of Item 5, "Minimum Record Standards, Outpatients", Article VII, Rules and Regulations, to clarify meaning of "outpatient (followup) admissions"; and addition of sentence at end of second paragraph, outlining the exception to this policy regarding psychological and sociological testing programs conducted by NIMH in the Clinical Center Follow-up Department and in NIMH clinical laboratories at NIH.

Amendment No. 15., Revision of first sentence of Article VI, Section 2,B, "Combined Clinical Staff Conference"; entire revision of Section 2,C, "Audit of Medical Care, Combined Staff, Clinical Center".

Amendment No. 16., Addition of sentences to Article V, Section 6,C, paragraph No. 6. "Clinical Research", regarding pro tem approval of minor change in a previously approved research project.

Amendment No. 17., Revision of Article V, Section 6, C, item 8 to define the function of the Radiation Committee with respect to administration of radiation to normal control subjects.

Panel of Consultants

During the year 1960, there were 723 visits by medical care consultants to the Clinical Center. Eight medical care consultants have been added to the roster, the total number now being 165.

The system for calling medical care consultants has been satisfactory Institute staff members responsible for covering a particular specialty are designated as panel chiefs. If a staff member wishes the services of a

medical care consultant he makes known his desire to the responsible panel chief who then makes arrangements for securing the services of the consultant desired. In this manner a consultant can be used by more than one Institute during one visit to the Clinical Center. It is felt that the consultant program, as a whole, has worked out very well.

Physiological Monitor

In keeping with its mission of originating new hospital methods, the staff of the Clinical Center has developed an electronic device for monitoring the physiological functions of post-operative and acutely ill patients. Help with technical details and production of the finished product was obtained from Gulton Industries, Inc., Metuchen, N. J..

It was decided that the five signs most needed in following an acutely ill patient's condition are: (1) blood pressure; (2) pulse; (3) temperature; (4) respiration; (5) arterial oxygen saturation. Electronic techniques for measurement of these vital functions have been used individually in the various aspects of space medicine, but have never before been combined in one package. The central control system can monitor four patients simultaneously.

A great value of the physiological monitor is that it records all measurements taken. A"print out" circuit is part of the equipment and it gives a permanent printed record in alpha-numerical form for each patient at whatever interval is determined to be necessary. The form may be pasted directly into the nurse's notes on the patient's chart and thus saves the time necessary for transcribing.

The monitor has now been in use at the Clinical Center for just under one month. Early indications are that it is a valuable adjunct. It is not felt that this inovation will, in any way, reduce nursing hours necessary for the care of patients. It is an auxiliary aid for the nurse which allows her time to perform the functions that only a nurse can perform, and it should help in providing better and more efficient patient care.

Information Office

All public information releases concerning the Clinical Center are made by its Information Office. It manages all inquiries from communications media and aids reporters and editors of newspapers and magazines, science writers, and radio and television personnel in preparation of material for the lay public. Among the articles appearing in the lay press this year were:

"They Volunteer to Suffer," by Howard Simon.
Saturday Evening Post, March 26, 1960.

"They're Giving Their Emotions to Science," by Roland Berg.
Look Magazine, April 26, 1960.

"Medicine's Human Guinea Pigs," by Frank Henry.
Baltimore Sun, full page, magazine section, December 11, 1960.

"NIH's Bethesda Hospital Nation's Most Exclusive," by Victoria Stone.
Washington Post, May 25, 1960.

"Convicts Catch Colds to Help NIH Scientists," by Phil Thomas.
Washington Star, December 7, 1960.

Another important function of the Information Office is its editorial responsibility in connection with the publication of certain contributions to the professional literature. Examples of the past year are:

Combined Clinical Staff Conferences of the Clinical Center for The Annals of Internal Medicine:

"Primary Amyloidosis," Vol. 52, No. 3, March 1960.

"Problems in Differential Diagnosis," Vol. 52, No. 6, June 1960.

"Recurrent Pulmonary Disease in a Child," Vol. 53, No. 3, September, 1960.

"Unexplained Death in a Patient With Lukemia," Vol. 53, No. 6 December 1960.

"The Selection of Instruments for Major Operations," by Janet Fitzwater, R.N.
Nursing Research, Vol. 9, No. 3, September 1960.

"The Use of Medical Records in Research," by Gloria S. Burich.
Journal of the American Association of Medical Record Librarians, Vol. 31, No. 4, August 1960.

"Top Brass Should Follow the Golden Rule," by Jack Masur, M.D.
The Modern Hospital, Vol. 95, No. 2, August 1960.

"Television as a Tool in Postgraduate and Undergraduate Medical Education," by Murray C. Brown, M.D.
New York State Journal of Medicine, Vol. 60, No. 6, March 1960.

"Pharmacy - Central Supply Services," by Milton W. Skolaut.
American Journal of Hospital Pharmacy, Vol. 17, No. 4, April 1960.
Vol. 17, No. 6, June 1960.
Vol. 17, No. 11, November 1960.

"A New Membrane Filter," by William H. Briner and Milton W. Skolaut.
Journal of the American Pharmaceutical Association, Vol. 21, No. 5, May 1960.

"Suggested Criteria for Proper Labeling of Solutions Requiring Reconstruction," by Philip R. Hugill and Milton W. Skolaut. American Journal of Hospital Pharmacy, Vol. 17, No. 6, June 1960.

"Equipment and Technics for Sterile Dispensing of Ophthalmic Solutions," by Philip R. Hugill, Boris J. Osheroff and Milton W. Skolaut. Journal of the American Pharmacutical Association, Vol. 17, No. 9, September 1960.

"Nuclear Medicine Has Come of Age," by William H. Briner. American Journal of Hospital Pharmacy, Vol. 17, No. 6, June 1960.

"Radiopharmacuticals are Drugs," by William H. Briner. The Modern Hospital, Vol. 95, No. 2, August 1960.

"Development and Clinical Investigation of a New Oral Surface Anesthetic for Acute and Chronic Oral Lesions," by Irwin I. Ship, D.M.D., Anderson F. Williams, D.M.D., and Boris J. Osheroff. Oral Surgery, Oral Medicine and Oral Pathology, Vol. 13, No. 5, May 1960.

Moving Picture

"Serving by the Pool of Bethesda," a 17-minute documentary film for the recruitment of Normal Volunteer control patients. Written and produced by the Information Office in cooperation with the Communicable Disease Center, USPHS, Atlanta, Georgia.

Exhibits

"Plaster Casts and Splints in the Care of Patients with Rheumatoid Arthritis" - David M. Fried, M.D.
Annual Meeting of the American Medical Association, Miami, Fla., June 1960; and Clinical Meeting of the American Medical Association, Washington, D.C., November 1960.

"Selected Clinical Studies of the National Institutes of Health"- Clifton K. Himmelsbach, M.D.
Clinical Meeting of the American Medical Association, Washington, D.C., November 1960.

"Ancillary Radiological Devices" - Theodore F. Hilbish, M.D.
Meeting of the North American Radiological Society, Cincinnati, Ohio, December 1960.

Revision

"Current Clinical Studies and Patient Referral Procedures," USPHS Publication No. 284, revised and expanded, July 1960.

Visitor Program

Official visitors to NIH averaged 1,000 per month, and this constant flow of guests is cared for by the Special Events Section of the Information Office. Our visitors range from eminent scientists and researchers through the gamut of physicians, university and college officials, public health officials, and members of the professions ancillary to medicine. They come from every State in the Union, from every continent and, it might almost be said, from every country in the world. Each is well received and the details of visiting are programmed to suit individual requirements which are dependent upon the visitors' special areas of interest.

Another duty of the Special Events Section is to manage arrangements for meetings, including the NIH Winter Lecture Series. NIH also serves as a meeting place for national and international groups. Examples during 1960 were:

Meetings

Regional Conference for Montgomery, Prince George's and Southern Maryland Counties on Aging.

International Rehabilitation Seminar.

PHS Influenza Symposium Committee.

Army, Navy, Marine and PHS Reserve Officers: Program on current medical problems.

Annual Meeting of the Southern Society of Anesthesiologists.

American Dental Association Conference on Growth.

Council on Medical TV -- Institute for Advancement of Medical Communication.

Optical Society of America.

Medical Faculty of the Free University of West Berlin and representatives of the Benjamin Franklin Foundation.

International Anesthesia Research Society.

American College of Physicians Course in Cardiovascular Diseases.

Montgomery County Tuberculosis and Heart Association.

American College Public Relations Association.

Members of the Medical Faculty of the Free University of West Berlin.

American Association of Inhalation Therapists.

1960 Study Tour of the Clinical Center of the International Hospital Federation.

Montgomery County Mental Health Association.

Representatives of the Ministries of Health for England and Scotland.

Veterinary Division Group of the American Association of Land Grant Colleges and Universities.

Pediatric Travel Club.

Westchester County Surgical Society.

Scientific Session of the Alpha Omega Fraternity.

Soviet Delegations

USSR Delegation on Radiation Medicine.

USSR Delegation on Metabolic Diseases.

Soviet Group attending the Congress of Anatomy in New York.

USSR Heart Delegation.

Soviet Tourist Group of Engineers and Physicians.

USSR Cancer Delegation.

Distinguished Visitors

Rear Adm. Helio Lopez, President, Argentine National Atomic Energy Commission.

Dr. El Azmeh, Minister of Health of the United Arab Republic.

Ambassador W.M.Q. Halm of Ghana.

Mr. Sean Mac Entee, Deputy Prime Minister and Minister for Health and Social Welfare of Ireland.

Dedication of the Division of Biologics Standards Building by the King of Thailand.

His Excellency Louis Scheyzen, Ambassador of Belguim.

Dr. Gudmund Harlem, Minister for Social Affairs, Norway.

Mr. William Francis Sheahan, Minister of Health,
New South Wales, Australia.

Dr. Arne Tiselius, Physical Biochemist, Uppsala University,
Sweden. Nobel Prize Winner.

Dr. Ludo van Bogaert, President of the World Fereration
of Neurology.

Hon. Kashi Nath Gautnam, Minister of Health, Nepal.

Mr. Aire Oran, Israeli Ambassador to Venzuela.

Calendar Year 1960

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT

CLINICAL CENTER

CLINICAL AND PROFESSIONAL EDUCATION

CC-02
Serial No.

This organization is concerned with a variety of programs of professional education and other activities with educational implications for the Clinical Center and the NIH.

Clinical Fellows (Residents):

Two types of residencies are offered at the Clinical Center. Both programs are administered through this office.

The professional departments of the Clinical Center listed below are in a position to provide complete training in their specialty fields. This training is not only complete but includes research components not found elsewhere. These programs are outlined below:

Service	Approved	Residents Currently in Training
Anatomical Pathology 1/	3 years	6
Clinical Pathology 1/	3 years	6
Diagnostic Radiology	4 years	4
Oral Pathology	2 years	1
Periodontia	2 yrs.(approval pending)	2

1/ Combined 4 year program in Clinical and Anatomical Pathology is also offered.

The only significant change in these programs is the approval of the program in Oral Pathology.

The Institutes conduct residency training on their services in those instances and for such time periods as are warranted. The basic limitation upon these programs is the fact that the research mission of these clinical services tends to restrict the clinical material available. The minimum acceptable skills for participating in the research tends to exclude residents. It will be recognized that the training of Clinical Associates substantially equates with the training of residents elsewhere, differing only in its lack of approval by accrediting bodies and the somewhat restricted range of clinical material. Both Fellows and Associates receive training which is exceptionally strong in research methodology. The existing approved programs of Clinical Fellowship (Residency) are listed below:

Service	Institute	Approved	Residents Currently in Training
Internal Medicine	NHI	1 year	6
Neurology	NINDB	2 years	2
Psychiatry	NIMH	1 year	5
Ophthalmology	NINDB	2 years	1
Dermatology	NCI	1 year	1

Clinical Associates:

Since 1953, when the Clinical Center opened, the six medical Institutes have appointed young physicians to perform the duties ordinarily given to residents. These appointments were made to clinical services that could not qualify for approval of residency training without changing their operating practices. This office was not concerned with these appointments which were considered a part of the various Institutes' total provision of medical staff for research and medical care in the Clinical Center. Most of the Institutes require that these appointees have one or more years of training in residency before appointment and all of them provide a supervised program that includes considerable laboratory research.

When the CORD program of deferment under Selective Service was developed by the PHS, this office interested the Institutes in using this device to make future appointments. This stabilized staff patterns one or more years into the future, and also took advantage of the recruiting inducement that deferment for training and assured future duty with one of our Institutes offered.

Research Associates:

In 1957 the National Institutes of Health, through its Scientific Advisory Committee, initiated a program of training for young physicians designed to prepare them for investigative careers in the basic medical

sciences. The program is designed to combine some of the best aspects of full-time study in the basic sciences in a graduate school on the one hand, and post-doctoral fellowship training in a research laboratory on the other.

The Research Associates, appointed by the Scientific Directors of the individual Institutes, generally come to NIH after completion of their internship or first year of residency and stay for a two year period. During their tenure the Associates devote the larger portion of their time to laboratory research under the supervision of a preceptor. Research problems are selected so that the Associates can acquire breadth and perspective, encounter a variety of laboratory problems and learn a variety of research approaches rather than become specialists in one or two refined techniques.

The program consists of a series of formal lectures in the basic sciences related to medical research; a series of informal seminars covering biochemistry, physiology, microbiology, genetics and psychology; and a weekly Journal Club is held through the two year period, giving the Associates an opportunity to present reviews in fields of interest to them and to discuss the research problems they are working on in their own laboratories.

The CORD Program:

Both programs of training use the CORD mechanism. Deferments are arranged through this office's representations to the PHS CORD Committee through appropriate channels. The Chief participates in the CORD Committee's deliberations as a member. From the beginning, NIH requested that men be deferred for specific assignments in specific Institutes, a pattern that has now been made standard throughout the PHS deferment procedure.

The "Matching Program":

The National Institutes of Health Clinical and Research Associate "Matching Program" came into being to meet a variety of problems and needs. In 1958 we became aware of serious difficulties arising from the fact that increasing numbers of young physicians knew of the opportunities at NIH, and the desirability of our combination of deferment and concomitant research training for two years as a method of discharging Selective Service obligations. It became clear that a central mechanism for coordinating certain aspects of a selection process for these two programs was needed. This office agreed to supply the services necessary for this coordination and developed an operating plan in collaboration with the Clinical and Scientific Directors involved. The most important elements of the "Matching Program" are:

1. It provides an orderly, systematic method whereby the Institutes can appoint and defer future junior staff members without inter-Institute conflicts, and with the assurance that our relations with young professional people will be on a dignified, fair, competitive basis.

2. It provides a method whereby candidates for opportunities at NIH can be actively recruited by other programs in the Public Health Service in

the event they do not qualify or are not selected for appointment here.

3. It is designed to minimize unproductive processing of applicants and the consequent waste of NIH and Division of Personnel staff time.

The program for calendar year 1960 (for 1962 appointments) ran smoothly and according to the schedule set up at the beginning of the year. All six of the medical Institutes participated in the program and the NIDR participated for the first time. The other two Bureaus of the PHS actively recruited from the group of applicants that were not selected by NIH. About one-quarter of our rejected candidates accepted positions with other PHS programs. A statistical report on the program is attached.

Late in the year it was requested by the Clinical and Scientific Directors that this office write a set of "ground rules" for the Matching Program to be distributed to all persons in the various Institutes involved with the program. The procedures for the program and the calendar for next year's program are set forth on the attached schedule.

Activities in Support of Clinical Education in the Clinical Center:

This office provided a number of staff services to the Education Committee of the Medical Board of which its Chief is Chairman. This committee conducted 10 Combined Clinical Staff Meetings and Clinicopathologic Conferences. A list of these programs which are designed to provide inter-disciplinary and inter-institute exchange of professional knowledge follows:

Date	Kind	Topic	Attendance
1/28/60	CCSM (Night)	Recent Developments in Hypertension	306
2/11/60	CPC	Unexplained Death in a Patient with Leukemia	107
3/10/60	CCSM	Techniques of Open Heart Surgery	151
3/31/60	CCSM	A Preparatory Study of Clinical Research Methodology in a Mental Hospital Ward	46
5/12/60	CCSM	Cystic Fibrosis of the Pancreas	122
6/9/60	CCSM	Dental Caries	98
9/8/60	CPC	Lymphocytic Lymphoma	88
10/20/60	CCSM	Ecology of a Newly Recognized Common Respiratory Agent-RS Virus	97

11/17/60	CCSM	Energy Metabolism and Its Relationship to Cancer	147
12/8/60	CPC	Mitral Regurgitation	60

The Chief of Clinical and Professional Education attended the Congress on Medical Education and Licensure sponsored by the American Medical Association and the Meeting of the Association of American Medical Colleges for the purpose of stimulating applications for training at the Clinical Center and to interpret our programs to Deans and other Medical School officials. This also served to keep him in close touch with recent developments in Graduate Medical Education.

The Education Committee met 11 times during the year to recommend residents for appointment, plan staff meetings and to select four meetings which should be submitted to the Annals of Internal Medicine for publication. A very active subcommittee made the primary selections for publication and supervised the editorial work on these manuscripts. This represented a major contribution of time by the Editorial Subcommittee Chairman, Dr. C. Gordon Zubrod.

Staff support for all staff meetings was supplied by this office.

Training Outside the Service:

There has been a significant increase in the number of persons from NIH applying for training outside the PHS during 1960. NIH employees are trained under two separate authorities: Commissioned personnel under the PHS Act; and Civil Service personnel under Public Law 85-507.

Passage of Public Law 85-507 in 1958 gave a new impetus to the recognition of needs and demands for training at NIH. The training of our staff within and without the Federal Government is becoming a major activity, and almost a program within itself. The growth of this activity, with its financial implication and its impact upon research programs, is forcing a review of policy and the development of mechanisms for evaluation of justification for training and benefits from training. Within the legal framework of PL 85-507 and the PHS Act, NIH expects to develop sound educational methods for improving the effectiveness and efficiency of its staff. As a result of the passage of PL 85-507 this office was given the added responsibility of reviewing all applications for training of Civil Service personnel as well as for Commissioned personnel. Training given NIH personnel during 1960 almost doubled that of the previous year. 349 employees were approved for training at facilities outside the Federal Government.

NIH-USDA Graduate School Program:

The program of night courses conducted by NIH-USDA Graduate School Program continued to be of great interest to our employees and those of nearby agencies.

In the spring semester 260 persons registered for the 18 courses that were given; in the fall semester 555 persons registered for the 24 courses that were given.

It is our judgment that the registration figures do not indicate the full extent of the demand for this type of education. Inquiries received by this office indicate that a broader curriculum would attract an even larger number of students.

The trend toward scientific courses noted in previous reports continued in 1960. Interest in courses in administration and the liberal arts continues to decrease.

In 1959 the Scientific Advisory Committee stimulated the organization of a nonprofit corporation called "The Foundation for Advanced Education in the Sciences, Inc." It formulated a proposal for consideration by NIH and the Surgeon General which provided for the operation of this program by the Foundation, and discontinuance of the present arrangement with the Graduate School in order to obtain a more flexible and effective administration for the night school program. This proposal is still under study at the present time.

Commissioned Officer Student Training and Extern Program (COSTEP):

The COSTEP Program entered its fifth year of formal organization in 1960. This program is designed to introduce young professional students to the PHS at an advanced stage in their predoctoral training through providing them with employment affording an opportunity for professional growth. The Chief of Clinical and Professional Education served as Chairman of the PHS COSTEP Committee for a 5 year period ranging from its organization in 1956 through 1960.

In the summer of 1960 seven Institutes and one Division employed 32 medical, 6 dental, 3 scientist, 3 veterinarian, and 2 engineering students. COSTEP operates year-round. The number of students employed during periods other than the summer months, though small, has steadily increased. This fall and winter 3 medical, 1 scientist, and 2 engineering students have been on duty.

The medical students this year were drawn from 20 U.S. schools of medicine.

Evaluation schedules completed by students and preceptors and an analysis of other program records has yielded information of specific interest which is presented below:

A. Table I

DATA ON CLINICAL ASSOCIATE AND RESEARCH ASSOCIATE APPOINTMENTS

Year Applied	Appl. Con.[1]	App'ts.[2]	COSTEP Con.	COSTEP Appt.
1956	-	-	1	1 for 1957
1957	-	-	-	-
1958	145	73	3	1 for 1960
1959	172	58	13	3 for 1961
1960	180	65	10	5 for 1962
TOTAL	497	196	27	10

1/ Includes COSTEP Applicants

2/ Includes COSTEP Appointments

Note: Figures for 1958 and prior years are provisional. Records not centralized in this office until 1959.

B. Table II

COSTEP - CLINICAL FELLOWS (RESIDENTS)

Year Applied	No. of COSTEP Applicants	No. of COSTEP Appointments
1957	1	1 for 1959
1958	1	1 for 1961
1959	1	1 for 1962
1960	1	1 for 1962

C. Forty-three per cent of the medical students indicated an interest in a PHS internship.[1]

D. Seventy-one per cent of the students expressed a desire for active duty following graduation.[1]

1/ Based on 28 evaluation questionnaires returned by medical students.

E. Eighty-six per cent of the students indicated they had other opportunities for professional appointment.1/

F. One hundred per cent of the students felt that their work experience would be valuable to them as they continued their professional education.1/

G. Sixty-five per cent of the preceptors reporting felt that their students (a total of 20) worthy of a special recruitment effort.2/

H. Eight evening seminars were held by NIH scientists primarily for the medical, dental, veterinary medicine, and Ph.D. candidates in the basic and biological sciences. COSTEP students assigned to other PHS Bureaus were extended invitations. In addition the seminars were open to science students employed under the Civil Service summer program. Usually six to eight NIH staff members were present for each session.

I. Inactivation interviews were held with the bulk of the students.

J. COSTEP Study - Since COSTEP completed 5 years of operation in 1960 this office felt that it was practical and timely to request that a study of the program be made. Such a request was initiated, approved by the PHS COSTEP Committee and forwarded to the Deputy Chief, Division of Personnel. Results of the study are not yet available.

The program continues to have the problems of limited space (many scientists indicate they would like to have a COSTEP student but have no physical space in which the man could work) and budget. On the latter item, COSTEP positions, unfortunately, are usually in direct competition with regular positions, equipment needs - in effect with all of the operating needs of the laboratory.

Orientation Programs:

NIH Orientation (Compass) Course

In 1960 four courses were attended by a total of 802 NIH employees. A Question & Answer Period for Commissioned Officers (primarily for new C.O.'s, but also open to veteran officers) was held as an integral part of each course. Serving as resource people for these meetings were representatives from OSG-PHS Personnel, NIH C.O. Unit, and the Clinical and Professional Education Branch-CC.

1/ Based on 28 evaluation questionnaires returned by medical students.

2/ Based on 31 evaluation questionnaires returned by preceptors of medical students.

Seventy-one officers attended these sessions. A number of administrative personnel interested in rules and regulations pertaining to the Corps have also attended.

PHS Management-Orientation Course

The NIH segment (one day of a five day program for C.O.'s and GS-9 and above nominated by their respective Bureaus) was presented on the following dates: March 22, June 7, September 27, and November 15. Approximately 55 persons attended each program.

Special Orientation Programs:

During the year seven program were held. These are listed below:

Date	Program	Attendance
3/14/60	Meeting for C.O.'s Planning to Inactivate in July	69 (Includes 14 Admin.Personnel)
4/2/60	Johns Hopkins - Post Doctoral Fellows (Planned & Executed with Spec.Events)	30
6/13/60	FIDB - NCI Lab. - Hagerstown, Md.	35
6/23/60	High School Science Teachers	19
7/7/60	Commissioned Officers Reporting to NIH (Primarily for physicians with patient care responsibilities)	95
7/12/60	COSTEP and Civil Service Summer Employees	78
7/27/60	COSTEP-Visit to PHS Hospital - Baltimore	23

PHS Orientation for New Employees

Approximately one-half day of this two-day program is spent at NIH. Following a general orientation and tour of the Clinical Center, conducted by the Special Events Section, the group is addressed by the Chief of Clinical and Professional Education or a senior staff member regarding the overall mission of this Bureau. Five such sessions were held in 1960; the average attendance for each session was 37.

High School Science Teachers Chemistry-Physics Institute:

This eight week program for high school teachers of science sponsored by American University, through a grant made by the National Science Foundation, was conducted for the fifth year in 1960. Nineteen teachers from twelve states, the District of Columbia, and one foreign country (India) reported to NIH on June 23. The following NIH Institutes and Divisions participated in the program: NCI, NHI, NIAID, NIAMD, NIMH, DBS, DRS. The teachers worked in NIH laboratories (on a non-pay basis) from 10:00 a.m. to 5:00 p.m. every Wednesday, Thursday and Friday. Evaluation of the program by both the teachers and the preceptors was favorable.

Counseling and Consultation:

In addition to counseling of young professional men relative to the Clinical Fellow, Clinical Associate, and Research Associate programs; there is student counseling - ranging from precollege level to graduate level - estimated per annum: Interviews - 110; telephone and written inquiries - 85. Counseling professional staff regarding their educational problems averages 65 individuals each year. This office is also consulted on a variety of educational activities ranging from problems in establishing educational programs in other agencies to preparation of educational materials for NIH use or by other Bureaus of the PHS.

Miscellaneous:

The annual report on Residency Training was prepared for the American Medical Association. The Interns and Residents Report was also prepared for the AMA.

The data on NIH residency programs was prepared for inclusion in the PHS announcement on clinical training.

One hundred and twenty-nine physicians drawn from the metropolitan area returned evaluation questionnaires on the January 28 evening CCSC - "Recent Developments in Hypertension". Seventy rated the meeting as excellent; forty-eight rated it as good.

Approval was secured from the specialty boards for 2 residency training programs; Hematology and Oral Pathology.

Television Unit:

The Clinical Center Television Unit conducted its experimental work during 1960. A second technician was added to the group in March. In July the third technician was employed. In October the fourth budget person was employed. This is a minimum personnel complement for the basic elements of our system.

A Television Technical Training Program was started late in the year in order that the new technicians might be given better theoretical basis for their work and to train some people in related areas in basic television techniques. The bulk of the instruction was provided by Chief of Television Engineering Frank Vanaman. Some sessions were taught by Mr. Willard Whitehouse. In addition to a photographer from Mental Health and a motion picture photographer from Photographic Services, DRS, most of the sessions were attended by Dr. Frederic Bartter who evinced a special interest in this area.

Equipment

The basic black and white television equipment complement was completed in July of this year, providing the unit with the resources we originally contemplated for the Clinical Center system. This included the replacement of some outmoded control room equipment, as well as the addition of the third black and white camera and a large screen projector. The latter unit relieved us from the necessity of borrowing projection equipment for black and white programs.

Video Tape

For about four months during the past year the Ampex Corporation loaned NIH a video tape recorder in order that we might evaluate this tool for medical education and scientific research. The value of this instrument in medical communication was demonstrated thoroughly, and the work with tape was enthusiastically accepted by scientists and administrators who participated in some of these ventures. The projected test of the use of this instrument in research and for the making of clinical records was not tested, due to the fact the machine was recalled by the owners before the end of the year. We feel quite certain of its usefulness in recording from the X-ray to the intensifier for both clinical and research purposes. It was the consensus of all who were familiar with the tests that this piece of equipment is badly needed and should be purchased at the earliest possible date.

Pulse Distribution System

Two X-ray image intensifiers were purchased by NIH units during 1960. It was necessary that these units be supplied with standard RETMA syncronizing pulses if the television portions of the systems were to be brought to broadcast television standards and integrated with other program material. A policy decision was reached that these pulses should be supplied centrally from the television control room, as it was believed that this kind of equipment will be increasingly used at the Clinical Center and the maintenance of individual generators could become a serious problem. Arrangements were worked out with

Dr. George Williams whereby his sync generator could be used centrally and in parallel with an existing generator in the control room. Amplifiers were bought that would make it possible to feed the X-ray equipment and Dr. Williams' ultro-violet video microscope simultaneously. Cables have been installed to the various points to be served and the service is ready to be connected as soon as the Picker Company completes its work. It is planned to supply 24 hour-a-day service and to maintain a standby generator which can be manually switched in event of equipment failure.

Patient Television Distribution System

The Patient Television Distribution System had not operated to our satisfaction, nor had its maintenance been adequate. During the year we arranged for a complete overhaul of this system by the Spencer-Kennedy Laboratories who had supplied the original equipment. Three Baltimore channels were added to the existing four Washington channels in order to improve the service to patients. A great deal of assistance and design work by the Television Unit was involved. The system is now awaiting permanent re-packaging by PEB.

Surplus Property

Changes in the regulations governing GSA and military handling of military surplus came to our attention in January of 1960. After several visits to installations where surplus was available, the staff became sufficiently familiar with procedures and the availability of certain kinds of equipment to permit the working out of a new joint procedure for acquiring a large amount of valuable materiel. In brief, representatives of about 12 laboratories and operational units were called together to arrive at an estimate of their needs for electronic expendables. These were consolidated into a single listing. Arrangements were worked out to provide transportation from a central fund, and for joint presentation of these needs to central GSA utilization officers in the regions. In the early stages of the program all of the materiel was delivered to the Television Unit which, in turn, distributed it to the various users on the basis of their declared needs when the pool was developed. Late in the year this role was paralleled by the Technical Development Unit in Mental Health. Still later in the year Surplus resumed working with individual units. During the period of centralized operation most of the participating units acquired a large percentage of their component needs for the next year or two through this device. The saving in money (all materiel was transferred without reimbursement) was significant. The saving in manpower time, by eliminating our usual need to hunt for components in other laboratories when a piece of equipment failed, and the loss of professional and technical time in small lot ordering of emergency items was considerable.

By direct visits to sources, and in collaboration with our Surplus Property Unit, the Television Unit acquired a great deal of expensive electronic equipment which was needed, but which would otherwise not have been available. A great deal of this equipment was also acquired and distributed to related laboratories. While the original dollar value of the material acquired is not available to us at the moment, we are certain that this effort represents a

saving to NIH in the neighborhood of $100,000 which will be realized over the next year or so.

New Surgical Wing

In July we acquired the basic equipment necessary to construct a control console for the New Surgical Wing, and in the succeeding months this unit was completed exclusive of the color control equipment which will have to be purchased. From time to time during the year consultations have been held with the architects and with our own Research Facilities Planning Branch regarding the basic television facilities for this new building. Some experimental work has been done on the development of new optical systems needed for this building. Considerable discussion of potential camera designs and exploration of new developments that might bear upon these has occurred. Actual design work has been deferred in view of the fact that the building is not to be completed for some time and because of the pressure of other work in the Television Unit. We feel that the building will have to reach the stage where on-the-spot testing of equipment can be conducted before the equipment complement can be determined.

Television Programming Operations

We have conducted two experiments with the medical communication aspect of television, and have also conducted use of television in those situations in which its value has been demonstrated. Some of these activities are outlined below:

Compass Courses for NIH Personnel:	Total of four programs
PHS Senior Level Orientations:	Total of four programs
Council on Medical Television:	A series of programs lasting 2 days
Special Orientation for Clinical & Research Associates:	One program
Special Orientation for COSTEP:	One program
American Pharmacy Association:	A one-hour program between NIH and the Shoreham Hotel
International Hospital Federation Study Tour of Clinical Center:	One program - trilingual
Television Experiment in Education-Communication:	A special program in color between NIH and PHS downtown offices
Dyer Lecture:	One program - coverage for overflow crowd
Russian Tourists:	One program

Hospital Planners:	Demonstration of TV equipment - Marconi Camera, GPL Projector, Video Tape. Patient presented.
Re-runs of Video Tapes and Kinescopes:	26 showings of programs previously recorded for visiting groups, professional groups, and internal staff education.

Application of Television for Biological Research

We have conducted our work in this field despite the increasing demands on the unit originating from the activities outlined above. Six papers and one exhibit resulted. The exhibit, a joint one with the Ophthalmology Service at Walter Reed Hospital, was well received by the Academy of Ophthalmology and Otolargngology where it was awarded a third prize among the scientific exchange and a third prize for an exhibit design.

The papers are listed below:

Television as a Tool in Postgraduate and Undergraduate Medical Education. Brown, Murray C. Presented at Open Meeting of American Writers' Assoc., N.Y.C., April 30, 1959. N.Y. State Journal of Medicine. 60:6, March 1960.

Dynamics of Vascular Pathology in the Human Retina. A Case Report. Brown, Murray C.; Whitehouse, W. C.; Longfellow, Don; Davis, Sterling. Anat. Rec. 136: 354, 1960.

The Choroidal Circulation; A New Vascular Bed for Study of Microcirculation in the Intact Albino Rabbit. Brown, Murray C.; Warner, Louise; Whitehouse, W. C. Anat. Rec. 136: 354, 1960.

Color Television Photography of the Fundus Oculi. Lowrey, Austin; Rich, Richard; Brown, Murray C.; Whitehouse, W. C. American Academy of Ophthalmology and Otolargngology. 1960.

Techniques for Studying Microcirculation in the Eye. Brown, Murray C.; Warner, Louise. Anat. Rec. 138:399, 1960.

Electronic Image Processing; An Approach to the Study of Choroidal Vessels. Warner, Louise; Brown, Murray C. Bibliotheca Anatomica. 1960 (In press)

In addition we have conducted our work with Dr. Baldwin on the high frequency of radiation of primates. We have also worked with the Bausch & Lomb Optical Company on development of a new video black and white ophthalmoscope which, it is thought, would be of considerable value in clinical situations and for teaching. The design requirements for an improved version of the color television ophthalmoscope have been determined. We will shortly confer with the Leitz Company regarding the design of a prototype. It is hoped that this instrument will permit quantitative determination of physiological and biochemical changes in the retinal circulation.

Future plans for the manpower resources of this unit are being more and more absorbed by the uses of television in situations where its value has been demonstrated, leaving little time for investigative activities. This situation will not improve. Two technicians can be added in Fiscal 1962 but they will be absorbed by expected increase in activities, including the design of the New Surgical Wing facilities and their subsequent operation.

We have formulated a proposal that the Clinical Center System be used as the foundation for a more ambitious exploratory program to be supported by an Institute or Institutes. This program would have two aspects: one related to expansion of our investigations and applications to television and related technologies to medical research, and the other an expansion of our explorations for uses of television in biomedical communication. This proposal is being considered by the Clinical Center and by the NHI at the present time. In the meantime, the opportunity to add the two additional technicians projected for Fiscal 1962 exists at the present time. We have found it difficult to find competent personnel at Civil Service rates. Some very competent people are available at this time due to the redefinition and limitation of Walter Reed's television progrm to medical television. This has resulted in a reducation-in-force. We feel that funds should somehow be found for the employment of these people at the earliest possible date.

Over-the-Air Transmission and Cable Transmission of Television Information

The Television Unit has explored various means for inter-Institute communication with television signals over the past few years. This year we acquired a small broadcast type transmitter for use on UHF channel 20. Under experimental license from IRAC we have quietly conducted transmission experiments with this device. Application for a permanent assignment of channel 20 has been made. It is hoped that these experiments will demonstrate that this is an inexpensive and sufficiently private means of communication between NIH units and two other points to permit it to be quite useful to our mission.

For a long time the military medical installations in this area have wanted NIH to join the medical education television network which they established subsequent to our pioneer studies in 1957. We have felt the cost of microwave services was excessive despite the desirability of cooperating in this inter-institutional venture. A less expensive and also somewhat limited means of joining the network was the installation of coaxial cable between NIH and NNMC, which has microwave service to Walter Reed, and through that installation to the remainder of the network. Late in 1960 cables were installed to Navy, and these will be in use early in 1961.

Summary and Recommendations

The program accomplishments indicate that 1960 has been a productive year for this project. Certain needs have been identified:

1. A video tape recorder should be purchased at the earliest possible date. It is estimated that the machine that we have been using on loan can be acquired for $40,000.

2. Two technicians should be added to the unit at once, while the opportunity to find good people is at hand.

3. The more ambitious undertaking in medical communications and medical research which is under consideration should be implemented.

4. The continuance of our replacement of obsolete equipment is essential. If the Fiscal 1961 actual rate of $2500 in excess of the operating budget is made available this year it will suffice.

Attention is again called to the need for funds for the purchase of color television equipment for the New Surgical Wing. The cost of the initial units can be expected to be approximately $100,000 and the total requirement will be about $150,000.

12/20/60

STATISTICS ON 1962 CLINICAL AND RESEARCH ASSOCIATE "MATCHING PROGRAM"

Total Inquiries		1,219+
Non Eligibles		49
Applications Sent		450+
Files Established		201
Withdrew		6
Rejects before Selections by Institutes		15
Considered for Appointment		180
Interviews Arranged		686+
Clinical & Research Associates Appointed		
	1962	61
	1961	5
	1963	2
	1964	2

The following tabulation is a representation of the Medical Schools from which candidates applied and from which we appointed candidates.

SCHOOL	APPLICANTS	APPOINTEES
Stanford Univ. Sch. of Medicine	3	2
Univ. of Calif. Sch. of Medicine	1	
Yale Univ. Sch. of Medicine	5	3
Georgetown Univ. Sch. of Medicine	3	
George Washington Univ. Sch. of Medicine	2	1
Emory Univ. Sch. of Medicine	1	
Chicago Medical School	2	
Northwestern Univ. Medical Sch.	3	1
Univ. Chicago Sch. of Medicine	4	
Univ. Illinois College Medicine	2	
Indiana Univ. Sch. Medicine	1	
State Univ. Iowa College Medicine	1	
Univ. Louisville Sch. Medicine	2	1
Tulane Univ. Sch. Medicine	1	
Johns Hopkins Univ. Sch. Medicine	12	6
Univ. of Maryland Sch. Medicine	3	
Boston Univ. Sch. Medicine	4	1
Harvard Medical School	31	18
Tufts Univ. Sch. of Medicine	3	1
Univ. of Michigan Medical School	3	1
Univ. of Minnesota Medical School	2	1
St. Louis Univ. Sch. Medicine	1	
Washington Univ. Sch. of Medicine	14	8
Univ. Nebraska College of Medicine	1	
Albany Medical College	2	
Univ. of Buffalo Sch. Medicine	5	1
Albert Einstein College Medicine	5	1
Columbia Univ. P&S	13	7
Cornell Univ. Medical College	5	

SCHOOL	APPLICANTS	APPOINTEES
New York Medical College	1	
NYU College Medicine	9	2
State Univ. New York-Downstate	7	1
Univ. of Rochester Sch. Medicine	4	1
State Univ. New York-Upstate	4	1
Univ. North Carolina Sch. Medicine	2	1
Duke Univ. Sch. Medicine	4	1
Bowman Gray Sch. Medicine	1	
Univ. of Cincinnati College Medicine	3	
Western Reserve Univ. Sch. Medicine	1	1
Ohio State Univ. Col. Medicine	1	1
Univ. of Oklahoma Sch. Medicine	1	
Univ. Pennsylvania Sch. Medicine	8	1
Univ. of Pittsburgh Sch. Medicine	4	1
Univ. Tennessee College Medicine	1	
Vanderbilt Univ. Sch. Medicine	2	1
Univ. Texas Southwestern Medical Sch.	1	1
Univ. Texas Medical Branch	1	1
Baylor Univ. College Medicine	1	
Univ. Virginia Sch. Medicine	2	2
Medical College of Virginia	5	1
Univ. Washington Sch. Medicine	1	
Univ. Wisconsin Medical School	2	

The following tabulation is a representation of Hospitals at which candidates and appointees served their internships and residencies.

	INTERNSHIP		RESIDENCY	
HOSPITAL	APPLIED	APPOINTED	APPLIED	APPOINTED
Univ. of Alabama Hospital	1	1	1	1
Los Angeles County General	4	2	1	1
Univ. of California, Los Angeles	1	1	1	1
Univ. of California, San Francisco	2	1	1	
San Francisco General	1			
PHS Hospital, San Francisco	1	1		
Wadsworth VA Hospital, Los Angeles			1	1
Children's Hospital, Los Angeles	1			
Palo Alto-Stanford Hospitals				1
Cedars of Lebanon			1	1
Univ. of Colorado	2	1	2	
Grace New Haven	7	2	3	2
Greenwich Hospital	1		1	
West Haven VA Hospital			1	
George Washington	1			
Georgetown Univ. Hospital	1		1	
D. C. General			1	
Emory University Hospital			1	
Grady Memorial Hospital	1		1	
City Hospital, Columbus, Georgia	1			
Univ. of Illinois, R&E	5	2	1	
Cook County, Chicago	1			

HOSPITAL	INTERNSHIP		RESIDENCY	
	APPLIED	APPOINTED	APPLIED	APPOINTED
Evanston Hospital			1	
Univ. of Chicago Clinics	1		1	
Indiana Univ. Medical Center			2	
Univ. of Iowa Hospital	1			
Menorah Medical Center, Kansas	1			
Louisville General Hospital				1
Univ. Hospital Baltimore	3	1	2	2
Johns Hopkins	15	4	9	6
PHS Hospital Baltimore	2			
Baltimore City Hospital			1	
Massachusetts General	10	9	4	9
Peter Bent Brigham	5	2	1	2
Boston City Hospital	11	5	2	3
Children's Medical Center, Boston	2			
Mass. Mental Health Center			1	1
Massachusetts Memorial	2	1	1	
Beth Israel, Boston	6	1	2	2
Boston Floating Hospital	1			
Univ. of Michigan	1		2	2
Wayne County Hospital, Michigan	1			
Butterworth Hospital, Grand Rapids	1			
Univ. of Minnesota	2	1		1
Mayo Clinic			2	
Barnes Hospital	11	6	4	6
St. Louis Children's Hospital	1			
Jewish Hospital, St. Louis	1		1	
Bellevue	11	3	6	2
Memorial Center	1		1	
Strong Memorial	4	1	1	
Mt. Sinai, New York	4	1		
Maimonides Hospital			1	
Montefiore Hospital	3	1	3	
PHS Hospital, Saten Island	2	1		
St. Lukes, New York	1	1		
Long Island Jewish Hospital	2		1	
Presbyterian Hospital, New York	8	6	3	7
Bronx Municipal Hospital Center	4	2	1	3
New York Hospital	3	1	2	1
Francis Delafield Hospital			1	1
Brooklyn Jewish Hospital	1		1	
State Univ. of New York, Syracuse	1			
Kings County, Brooklyn	1			
North Carolina Memorial	1	1	1	2
Duke	6	3	3	4
Univ. Hospitals (Western Reserve)	2		1	
Cleveland Metropolitan General	2			
Ohio State Univ. Hospital	3	1		1
Univ. of Oklahoma	1	1	2	2
Providence Hospital, Oregon	1			
Univ. of Pennsylvania	2		2	2

HOSPITAL	INTERNSHIP		RESIDENCY	
	APPLIED	APPOINTED	APPLIED	APPOINTED
Children's Hospital, Phila.			1	1
Philadelphia General	1	1		
Univ. of Pittsburgh	3		1	
Jefferson Hospital	1			
Fitzgerald-Mercy, Lansdowne	1			
Institute of Pennsylvania Hospital			1	
Vanderbilt Hospital	3	2	1	2
John Caston Hospital	1		1	
Parkland Memorial Hospital, Texas	1			
John Sealy Hospital, Texas			1	1
Salt Lake County General Hospital	1			
University of Virginia Hospital	5		1	1
King County Hospital, Seattle	4	2	1	1
Univ. of Washington	1		1	
Univ. of Wisconsin Hospital	1			
Wisconsin General	1			
Hospital for Sick Children, London, England				1

12/21/60

SCHEDULE CLINICAL & RESEARCH ASSOCIATE MATCHING PROGRAM
for Calendar Year 1961

December & January	Complete follow-up of current program; Revise Information Packet
February	Application materials available for candidates
March April May June July August September	Receipt & processing of applications
Sept. 15 thru Sept. 30	Request confidential statements from "Chiefs of Service" on all candidates with applications on file.
Oct. 1	Deadline for receipt of completed applications
Oct. 2-6	Review of last applications by Institutes
Oct. 9-27	Interviews
Oct. 27-Nov. 1	Selections of appointees by Institutes
Nov. 2	Matching
Nov. 3	Institutes notified of results of matching
Nov. 6	Inter-Institute negotiations & final approval of matching results
Nov. 7	Notification of successful candidates for confirmation of appointment
Nov. 8-10	Notification of all candidates by letter; Release of unsuccessful candidates for recruiting by other programs; Notification of Division of Personnel, OSG, regarding action taken on candidates.
Nov. 13	Mailing of PHS Commission applications and CORD Forms; Follow-up on program

CLINICAL AND RESEARCH ASSOCIATE "MATCHING PROGRAM"

The National Institutes of Health Clinical and Research Associate "Matching Program" came into being to meet a variety of problems and needs. Its most important elements are:

1. It provides an orderly, systematic method whereby the Institutes can appoint and defer future junior staff members without inter-Institute conflicts, and with the assurance that our relations with young professional people will be on a dignified, fair, competitive basis.

2. It provides a method whereby candidates for opportunities at NIH can be actively recruited for other positions in the Public Health Service in the event they do not qualify or are not selected for appointment here.

3. It is designed to minimize unproductive processing of applicants and the consequent waste of NIH and Division of Personnel staff time.

PROCEDURES FOR OPERATION OF THE PROGRAM

I. Inquiries

Inquiries reach this office from many sources: These include Office of the Director, NIH; Director, Clinical Center; Office of the Surgeon General; Clinical Directors; Scientific Directors; Laboratory Chiefs.

II. Actions Taken on Inquiries

A. Candidates in which NIH is not interested are sent the following:

1. Letter outlining requirements for appointment at NIH and stating candidate does not qualify on one or more points.

2. Letter discouraging applicant who minimaly qualifies but will not successfully compete (candidate is advised of other opportunities in the PHS).

B. Candidates of definite interest to NIH:

1. Inquiries may be answered directly by Clinical or Scientific Directors if they so desire, or they may be forwarded to this office for reply.

2. If inquiries are answered by the Institutes it is requested that a copy be sent to this office, so that a file card may be established. As candidates may apply to several Institutes simultaneously the above is very important to avoid duplication and to maintain continuity.

3. It is also requested that all "Matching Program Packets" be mailed by this office rather than the Institutes.

4. A file card is established for every inquiry received.

5. All candidates for the Clinical & Research Associate programs will be required to complete the various information sheets contained in the Packet.

III. Actions Taken on Information Sheets (applications) Received

A. Upon receipt of the completed information sheets a file is established for each candidate.

B. When the file is complete i.e. letters of reference (3) and grade transcripts, the material is photocopied and sent to each of the Institutes which the candidate has indicated he would like to apply. A copy of the file is also sent to the Division of Personnel, PHS.

C. Attached to each candidate's file is a route slip asking for your action on the candidate; i.e. reject or arrange interview. This slip is to be returned to this office within two weeks of receipt of the file by the Institutes.

D. An additional letter of reference will be requested from the "Chief of Service" during the latter part of September. This will be duplicated and sent to the Institutes interested in interviewing the candidate.

E. Photographs and Grade Transcripts will not be duplicated with the files, but will be maintained in a master file in this office. They will be sent to the Institutes on loan upon request.

F. Deadline for receipt of completed applications is October 1, 1961.

IV. Interviews

A. All candidates will be notified by this office the date of their interviews and the Institutes who wish to interview them.

B. All interviews will be held during the three week period October 9 thru October 27, 1961.

C. All persons in the Institutes who will be interviewing candidates will be asked to inform this office the days of the week and the hours when they will be available during the above three week interview period. This information will be asked for sometime in July.

D. One half hour is allowed for each interview.

E. Each person interviewing candidates will be sent a weekly interview schedule marked off in half-hour blocks for each day. As interviews are arranged you will receive a slip giving candidate's name, date of interview and time. Please be sure that this information is

put on the weekly schedule.

F. Each candidate will be given a detailed interview schedule listing time, Institute, Interviewer and location of interview, when he reports to this office the day of his interview.

G. If you desire to have a candidate interviewed by another person within your Institute, please check his schedule before making arrangements.

H. If you think a candidate should be interviewed by a person or persons in another Institute please check with Miss Long before making these arrangements. This is necessary because: The candidate may have applied to this Institute; his file will have been reviewed by persons in this Institute; if the Institute is not listed on his interview schedule it means they are not interested in a person with his background and qualifications. If you still feel that the candidate should be seen please make arrangements thru the Clinical or Scientific Director of the Institute.

I. No candidate is to be offered a position at the time of interviews.

J. When the candidate has completed his interviews he will fill out a rank order sheet listing Institute and Laboratory or Section, if this is necessary, within the Institute.

V. Selection, Matching and Notification of Appointees

A. By November 1, Institutes will submit, to this office in writing, a rank order list of the candidates they wish to appoint. This list should contain at least twice the number of candidates needed for your positions.

B. The rank order lists of the Institutes will be matched with the candidates rank order sheets.

C. On November 3, Institutes will be notified by telephone of the results of the matching.

D. If an Institute has chosen a candidate for appointment and the candidate has not listed the Institute, or Section within an Institute, on his rank order list and has not been selected by one he did list, the Institute will be given this information and two courses of action may be taken:

1. The Institute can select another appointee.
2. The Institute may offer the position to the candidate to see if he is interested and will accept.

E. On November 7, the Institutes will, by telephone, offer their appointees the positions for which they were selected (subject to approval of their nomination for CCRD deferment if this is involved)

and ask for a verbal acceptance. As the Institutes wish the telephone call may be followed up with a letter of appointment or not.

F. If an "appointment letter" is not to be written, at the time of the telephone call the Institute should ask for a letter of acceptance from the candidate.

G. If an "appointment letter" is written the acceptance letter can be requested at that time.

H. Two copies of the appointment and acceptance letter on each appointee should be sent to this office.

I. Rejection letters to all unsuccessful candidates will be mailed on November 10.

J. On November 13, applications for Commission and Deferment Forms will be mailed to all successful candidates.

VI. Miscellaneous Information Re Program That Will Be Provided

A. Institutes will receive the following:

1. List of all candidates that were appointed

2. List of all candidates that were rejected

3. Statistical report on program

4. Notification by memorandum when your candidates have all filed their applications for commission and forms for deferment.

5. Notification by memorandum that candidates have been approved for a commission and selected for deferment.

Calendar Year 1960

PUBLIC HEALTH SERVICE, NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

PATIENT ACTIVITIES SECTION

CC:05
Serial No.

For the sixth year in a row, the number of Volunteers requested for medical research study has exceeded the supply. However, since the beginning of the year emphasis has been directed toward securing additional sources of Volunteers to meet the demand.

Gains were made in negotiating contracts with several agencies and the outlook is beginning to take on a new perspective. Since 1954, when the NIH had a contract only with the Brethren Service Commission and the Mennonite Central Committee, contracts have been completed with the following organizations, from whom we now can realize recruitment:

American University
Antioch College
Bennington College
Brethren Service Commission
City-County Clinic in Johnstown, Inc.
Home for the Jewish Aged, Phila. Pa.
Maryland University
Mennonite Central Committee
Montgomery Junior College
National Association of Retired
Civil Employees of Gaithersburg
Washington Academy of Sciences

The record shows that substantial gain has been made in the recruitment of Volunteers by utilizing the above sources. The projection into 1961 would indicate a more adequate supply of Volunteers to meet the several criteria established for specific studies.

As of now, 10 of the listed contractors have supplied Volunteers in one or more of the Institute projects. Therefore, this has resulted in a greater number of Volunteers participating in more studies than in any one year to date. They will have contributed a total of 58,272 days to the study effort and 650 Volunteers are identified with the total days.

Even though recruiting on a personal basis has been done since the inception of the program, it is obvious that since expansion brings increased responsibilities and problems, there will be a greater continuing demand for good public relations contacts with the contractors.

INSTITUTE	1960 TOTAL VOLUNTEERS	1960 TOTAL VOLUNTEERS DAYS
NIMH	116	9,495
NIAMD	20	1,786
NCI	5	383
NHI	50	3,644
NINDB	4	296
NIAID	76	1,324
NIDR	3	49
	274	16,977

RECREATION

Social Activities

The social phase of the recreation program involved parties celebrating holidays, as well as special parties; live shows, which included special performances by celebrities; motion pictures, most of which were of recent release; travelogs, lectures and spectator activities.

The quality of the variety shows has been considerably improved since talent has been made available by the Armed Forces. The Navy, Marine Corps, and Air Force have contributed outstanding talent both for live shows and for dance bands for our parties. The Elks, Scottish Rite Masons, the American Guild of Variety Artists, and the American Federation of Musicians presented outstanding programs for the patients. The parties and dances have met with such great success during the past year that it is now planned to have at least six big parties a year.

The adult movie attendance is about 65 per cent of patient population. Matinee attendance is about 95 per cent of the children census and about 30 per cent of the adult census.

Off reservation activities included securing of tickets for the Volunteers as well as for ambulatory patients to such events as plays, movies, lectures, etc.

Sports

Physical exercise activities of ambulatory patients and Normal Volunteers takes place in the 14th floor gymnasium. Some passive programming is conducted in the 4th floor solarium for Normal Volunteers, and in the 5th floor solarium for neurological patients.

Each morning between 10:00 a.m. and noon the gymnasium is reserved for the National Institute of Mental Health. The Patients Activities Section supplies the Mental Health units with equipment necessary to carry out their recreation programs. It does not direct or supervise the NIMH recreational program, but frequently assists at their request.

The gymnasium is open daily from 1:00 p.m. until 4:00 p.m. for patients needing specific supervision. After being medically cleared, patients from all Institutes may participate in this general interest program. The Social Service Department uses the gymnasium to give play therapy to patients of the National Institute of Arthritis and Metabolic Diseases from 4:00 to 5:00 p.m. The gymnasium staff provides assistance in the supervision of this program if requested.

The NIH Recreation and Welfare Association reserves the gymnasium for the use of employees between the hours of 5:00 and 7:00 p.m. Unless specifically reserved, the remaining evening hours are available to the National Institute of Mental Health.

The Patients Activities Section offers the following sports entertainment:

1. Patient participation in games such as basketball, volley ball, badminton, shuffle board, ping pong, etc.

   A scheduled game is included in the program occasionally, which offers a special event for spectator patients.

2. Community activities such as football games, baseball, hockey games, etc.

   The Patients Activities Section secures tickets for these events.

3. Supplies sports equipment to patients who participate in the following sports: golf, tennis, football, baseball, etc.

The major goals of the Section are:

1. To achieve maximum patient participation through the media of sports, social and spectator activities.

2. To have all patients participate in exercise to the full extent of their physical capabilities.

3. To offer such a diversified schedule of games that some segment of it will interest each individual patient.

The inadequate number of personnel assigned to this Section constitutes its major problem. Second only to this is the problem of space, which will increase steadily as more requests for recreation are received.

Patients' Library Unit

The long-range objectives of the Patients' Library Sub-Section are much the same as those outlined in previous reports: (1) It is proposed to inaugurate studies in Bibliotherapy; (2) To make improvements in Readers Guidance Therapy; (3) Wherever and whenever time permits, to attend meetings having to do with medical care and with coordinating efforts with others who have to do with direct patient care. This will make for better communications with such services as Occupational Therapy, Physical Therapy, and the Social Service Department. It will lead to saving time by avoiding duplication of work, and will permit us to coordinate schedules to avoid work interruption. (4) The librarian is studying reader-interest classification systems used in various libraries, and is taking steps to find what hospitals have used such a scheme and with what success. The system is especially adaptable to a small collection and would be a great help in readers' guidance services.

Other objectives we hope to implement in 1961 are:

1. Improved schedule of bookcart visits to nursing units.

Patients are kept so busy, often away from the nursing units, that it is difficult to find them in their rooms during the hours from 10:00 a.m. to noon, and much of the afternoon. Most nursing units have a rest period from 1:00 p.m. to 3:00 p.m.; there is a play period for children on the 14th floor every morning and afternoon; once a week there is a movie in the afternoon, etc. It is necessary to return to a nursing unit after lunch to try to see those missed in the morning, a procedure wasteful of time and energy.

The Librarian has met with the Chief Nurses, who have consulted with the Head Nurses and have sent memoranda to the Librarian suggesting the best times for visits. Experiments are being made with visiting during the noon hour when everybody is "at home," and during rest hours, when it is permissible to visit patients who are awake, and during early morning and late afternoon hours. New schedules will include special visits to nursing units where there are several children, and to special groups such as those isolated with infectious diseases.

2. Reading Night: A committee of four women from the PHS.

Commissioned Officers' Wives Club is assisting in planning a literary or reading program for patients. It is proposed to hold it every two weeks.

The committee will arrange for talent and work with the Librarian to insure interesting and varied offerings. This is an outgrowth of the National Library Week programs of the past.

3. Every effort is being made to acquaint other staff members with services the library offers.

At the request of the Chief, Chaplaincy Service, a study is being made of the use of religious books and materials. A record of requests of this type is being kept for the purpose of guiding future purchase of religious reading material.

Accomplishments of the past year may be listed as:

1. Talking Books for eye patients.

2. Books on records for other than eye patients.

3. Supplementing the school program.

4. Books in large print for the partially sighted.

5. Foreign language books.

6. Development of a small collection of paperback text and reference books to meet the requests of college students who are in the Normal Volunteer Program.

7. Expanded services to meet the needs of special groups of patients such as the Federal prisoners who are participating in the Normal Volunteer Program. Except for one group that read very little, the requests of these patients cover a wide range of subjects. Each is visited weekly by the bookcart and current periodicals are purchased for them weekly as needed.

8. The library cooperated in preparation for publication of a series of articles titled "The Furious Children and the Library," which reported and evaluated the library's role in the NIH project with emotionally disturbed children. The series appeared in "Top of the News," publication of the Children's Services Division of the American Library Association, and it is now available as a pamphlet.

9. To give better service to children, special bookcart visits are scheduled to nursing units where there are several. This allows a good selection of material, gives more personal attention to each child, and makes possible a story hour as part of the visit. A variation of the plan is to have groups of children visit the library for the same purposes.

10. National Library Week (April 3-9) was observed and the highlight was a Reading Night in the 14th Floor Auditorium. As in previous years, the library displayed mechanical reading aids and special materials, which library staff members explained and demonstrated to the visitors.

The major basic problems of the Patients' Library Sub-Section are inadequacy of space and number of personnel. These two problems are very closely interrelated.

The crowded conditions, lack of office and workroom space, storage space, and an adequate reading room, make maximum use of staff impossible. Although furniture has been rearranged and work is planned as efficiently as possible, there is no space where a staff member may carry on a project, whether it is cataloging, processing books, checking mail, selecting books, planning group reading programs, or anything else. There are constant interruptions to all tasks, due entirely to lack of space. Because of this there is no way to plan a normal work-flow and, as a consequence, it is not possible to use Gray Ladies and patient workers in much of the "behind the scenes" work which they could do well if there was a place for them to work, and an opportunity to have their work laid out and waiting for them.

For example, in loading bookcarts for the nursing units, much time is lost moving the carts out of each others' way, or squeezing past the cart to reach needed books and to load the shelves. This work could be accomplished in half the time if there were proper room in which to work. These conditions hamper the development of a first rate library program and the best utilization of staff. The physical condition of the library is costing an unnecessary outlay of time and money, and should be corrected in the interest of economy and efficiency. In summation of the space problem, let it be said that there is immediate need for (1) A Librarians office; (2) An outer office and workroom; (3) Storage space for bookcarts and reading aids; (4) Lockers; and, (5) A reading room at least the size of the present library.

With regard to staff, it is hard to be accurate in making an estimate because, given sufficient space in which to work, many more things could be accomplished than are presently done. It is quite probable that a staff of two librarians and a library assistant could do many of the things (such as attending unit staff meetings, conducting additional reading and discussion groups and story hours), which are necessary if the Patients' Library is to be an efficient part of the Clinical Center team, and coordinate its service with others. The addition of a professional librarian, perhaps a children's specialist, should be given prime consideration as the space problem is solved.

The most serious staffing problem is dependence upon volunteer help to carry out much of the scheduled activities. If the volunteers actually came every week except for, say, one or two months vacation, the library program could be augmented with many additional services. To make this point - out of a possible 234 working days in 1960, there were 100 days when no Gray Ladies reported for duty, and the librarians had to do their

work as well as their own, or cancel or postpone duties in order to meet the basic service of visiting each nursing unit weekly.

This absenteeism was due to understandable, legitimate causes. In many cases advance notice was given so altered work schedules could be planned in advance. The Gray Ladies are loyal, devoted women. They do their work well, are cooperative, and their presence in the hospital is important to the patients. The Clinical Center is greatly indebted to them for their many hours of work, for their patience and cooperation, and for their fine spirit. But the fact still remains that it is almost impossible to maintain a scheduled service to patients with volunteer staffing.

With regard to patient help, under present conditions it is not possible to utilize it properly, nor to offer the library as a therapeutic or rehabilitative part of their treatment in the way it should be done. Some valuable assistance has been received from patients, especially from Normal Volunteers who have been assigned to the library for several hours each day. But the training available and the type of work that can be planned for patients is limited because of the space situation.

The library has now reached its planned size of 4,000 hard-covered books. From now on, the same number of books will be withdrawn as is added to the collection.

Children's Recreation Unit

The Patient Activities Section provides a daily play program for the more than fifty children housed in the Clinical Center. However, inappropriate play areas indoors and total lack of outdoor areas present almost insurmountable obstacles to providing full service and efficient utilization of personnel.

The concept of custodial care must be discarded as inadequate and outmoded. Play time is the child's time to work, grow, and experiment with his world -- not an activity to send him to when he has nothing better to do. Ruth Hartley says in Understanding Children's Play that play experiences are so valuable to a child because they take the intermediate place between inarticulate, subjective impression, and the structured language, and the prescribed conduct of adult social communication. Furthermore, Erik Erikson suggests that a child uses play to make up for defeats, sufferings, and frustrations.... These statements from recognized experts have definite implications upon our program and problems here.

Pre-planning is essential in dealing with children in a play situation (or in any situation). To plan means to look ahead; yet because of the unsuitable physical layout, free play is about all the planning that can be done, and this can become a dangerous and frightening situation, instead of a happy and satisfying experience.

These children, because of their illnesses and absence from home, are anxious, insecure, and frightened already; so a good play situation should provide warmth, reassurance, normalcy -- and fun. With the present conditions, this is next to impossible. Play is restricted to the 14th floor and gymnasium. This large open area is difficult to supervise adequately because of walls and corners. For instance, Melanie, an active five year old leukemic in remission, has some problems with her home situation and is rebellious. When our backs were turned to put away the toys in the far-distant storage area, she slipped away and it took fifteen minutes to catch up with her. She had been riding up and down on the elevators all by herself. This same child had nearly fainted from fatigue when she was being returned to her floor on an earlier occasion.

All visitors tour daily through play time; during religious observances the chapel congregation walks through; mental patients come that way to use the gym; medical personnel hold meetings in the auditorium, and visitors to patients pound the piano. At least 60 per cent of these extraneous people stop and talk with the children. For example: Alice is well known at NIH. She needs, more than most children, a normal play relationship with other children. Yet when she was busily weaving an imaginary tale with another little girl and a doll, along came a visitor and a nurse. "Hi, Alice." "Hi". (Alice continued with her story.) "What's the matter? Can't you smile?" Frankly, when Alice shouted at the person in her inimitable way, her play supervisor was secretly pleased.

On the following pages is described equipment, schedules, structure, and program that can be carried out, if permitted, by administrative action on play space indoors and out. Only a limited program can be accomplished without this action. It is frustrating personally to see and know what might be done, and to be unable to attain objectives beneficial to everyone, especially the children.

These are the objectives of a play program seen from the Patient Activities Section's viewpoint:

1. Provide a meaningful play experience that can enrich a child's life, rather than custodial care.

2. Give children TLC in a normal play situation away from the medical aspects.

3. Allow for the release of tensions and hostilities through play, which would help the child cooperate with the necessary medical procedures.

4. Provide the nursing staff with insight to another side of the child's personality that can help, if there are problems on the nursing units.

With these objectives in mind, and accepting the need for structure, here is a possible solution: In the mornings, 9:30 to 12:00, have a nursery school group for every child under six years of age. In the afternoons, a school-age group should have the choice of OT and Patient Activities Section so they get both crafts and active play - perhaps an alternating schedule could be set up.

To achieve a good play program, children must be grouped with like ages because each age has its own way of using play time, toys, and equipment. Older children find younger children a nuisance, for instance, when they are trying to paint a picture or play monopoly, and a smaller child grabs the paint brush or upsets the monopoly board. Beyond this, young children use a different sort of paint and brush than older children, and it is not possible to have both of them out at the same time.

In many instances it is not physically safe to have smaller children in the vicinity of larger ones when they are letting off steam. For instance: John, Billy, and two Marks, all very active 7-9 year olds were riding as fast as they could possibly go on bikes and scooter, when slow-moving 3-year-old Alice wandered directly into their paths. Had it not been for the fast action of a Normal Volunteer, Alice would have been knocked down very roughly, and Alice is not supposed to get knocked about. This sort of near-accident is duplicated almost daily.

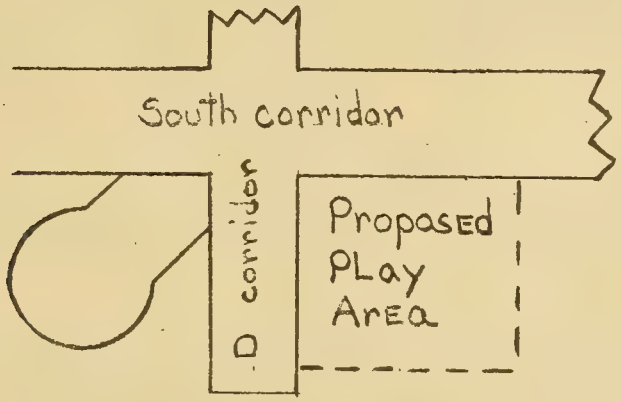
A structured setup can be worked out by weekly conferences with Occupational Therapy, Patient Activities Section, and others concerned with care of the children. A schedule could be devised which would give everyone some children to work with, avoid duplication of activity, and make sure each child had something to do. These meetings would further aid in work with the children because knowledge could be shared, and the more complete picture that can be had of a child the more can be done for him. Granted that Patient Activities Section is a secondary service in a setting where medical research is primary, still children's workers need to function as part of the team, rather than in isolation or working in a vacuum. More information is needed on each child than is now available.

The necessity for an indoor play area is well known and has often been discussed. An urgent need, and one that could be readily attainable, is an outdoor play area near the Clinical Center. Although these are sick children, they are very energetic at times and quite able to go outside if there were a place. Children do not like to go out for a "walk". They want a place to go and things to do when they get there. For instance, leukemic children in remission are almost normal, and may participate in normal play. Children with cystic fibrosis can, and so can those on metabolic studies not involving diets, etc. An outdoor play area would be as much used as any outdoor play area in any location, in any city. Any objection as to seasonal use can be answered by, "Why then ever build outside play areas anywhere?"

Any child gets rebellious and fretful if kept inside for long periods of time. All children need a place free from restrictions yet safe, as a well-planned play area would be. The children here on strange and often frightening routines would benefit; and so would the nursing staff, for the children would be easier to handle on the nursing units.

Since NIH is an international showplace, a well-designed, equipped, and used outside play area can be shown as another aspect of the unique approach to medicine here.

Any such area must be near the Clinical Center, and one area is that to the rear of the building bounded by D corridor and the South corridor; and marked recreation area on the master building plan. Nothing is in that area at present, and a playground could easily be arranged there.



Here, instead of a plumber's nightmare of pipes, arm-breaking swings, and chin-bumping see-saws, several beautifully designed pieces such as a play puddle, a ring roost climber, a helical side, plus a sand box, and a paved trike and bike riding area can be placed. In addition, there must be a storage shed, which could be used as a play house; an accessible bathroom (preferrably child-sized); a drinking fountain, and an outside telephone. The telephone would be for emergencies as well as making the area accessible so that the nursing units could call their patients. This play area can be equipped as expensively or as cheaply as desired, as long as thought goes into the planning. The most important thing is to have it, and fenced so there will be relative ease of supervision, even with a retarded child.

As a stop-gap, we have used the playground behind T-4 this summer, but this is unsatisfactory for several reasons: (1) It is too far for the children to walk; (2) It is a stop-gap, and that isn't good enough; for it is not facing the fact that there are always going to be children in the hospital, and they will always need a place to play outside. This need has been repeatedly pointed out, but there is still no sign of a playground.

As mentioned in a previous first report, we must have a good indoor play area that does not give the aspect of Grand Central Station; that has sufficient storage space available, and that has a piano. Other considerations are child-sized toilets and drinking fountains, and accessibility to the outside area. This is when a good nursery-type program can succeed. Such a program would include an extra large dose of free creative and dramatic play (given proper equipment and space, a minimum of adult intervention is needed). There would be a singing time, a story time, a group game or two. This is somewhat structured, but less than a normal nursery for it serves a different purpose. The real structure would lie in the worker's selection of the day's play material, which would vary according to her knowledge of the children. With a room that can be locked away from the public, materials for the morning's play can be set up the night before; thus eliminating waste of time in the morning. This can be done only by preplanning with the other departments about schedule.

In the same room, the pre-school equipment can be stored and the things for the older children brought out. What a program would be for children inside would vary definitely with their age and sex, because as children grow older they exhibit strong preferences for certain types of behavior and activities based on their sex. However, both groups would play outside on every nice day possible.

Over and above the day-to-day relationships and programs, entertainment is easily secured with the cooperation of various amateur and professional people around town. These provide a welcome change from every-day life, but cannot substitute for a daily program.

Several other points made previously bear repeating. For instance, there should be a blanket activity permit form filled out as part of admissions procedure; with a trip permit on the back so children may leave the reservation to go sightseeing, the zoo, or in the case of teen-age boys, to ball games. A mention of needed clothing such as sweaters, shoes, etc., would be good as pre-admission procedure. Parents tend to take home these items, and many children have come out to play in slippers. These activity forms, when returned to us, would also give us a file on patients. In conjunction with these trip permissions, if the motor pool could turn over to Patient Activities Section a station wagon, a great deal of red tape could be eliminated concerning trips.

Calendar Year 1960

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER
OFFICE OF THE DIRECTOR

CC-09
Serial No.

DEPARTMENT OF SPIRITUAL MINISTRY

PROTESTANT CHAPLAIN

In March, the Supervisory Chaplain, Mr. Andrew, and Dr. Himmelsbach discussed the survey, which Mr. Andrew conducted in 1959, of the Protestant patient population. On the basis of this study and on views based on his experience and that of others, Chaplain Andrew formulated a proposal which would involve a considerable increase in the chaplaincy activities of the Department. The discussion of this report resulted in the following understandings:

1) That it would be important to institute a continuing, scientifically sound appraisal of the patients' spiritual needs, which would be meaningful and useful to the proper administration of the Clinical Center and the Department of Spiritual Ministry;

2) That such a study requires a consultant who has considerable sophistication in the field of research design, methodology and analysis, in the general area of the humanities and social and behavioral sciences;

3) That the new chaplain position be utilized in these studies.

4) That the results of these studies and others will likely raise various other questions which will need to be investigated by sound scientific methodoly; and

5) That possibly, if this works out, consideration might be given in the future to a program of training of research associates in spiritual ministry.

These agreements were implemented in the following months. Dr. Urie Bronfenbrenner was consulted regarding the feasibility of the proposed use of scientific methods in studying the spiritual needs of patients. It was his opinion that the proposal looked promising. He recommended as a consultant the Rev. James E. Dittes, Ph.D., Professor at Yale Divinity School.

At this time, the proposed plan was also discussed with the Catholic and Jewish Chaplains. It was agreed that no unified research could be made by the Department as a whole, due to variations in religious traditions, dogmas, practices and premises. Individually, Rabbi Brandriss would be interested in research, if he had time from his full-time duties in the Synagogue. The Catholic Chaplain's program is not amenable to research.

PROTESTANT CHAPLAIN

It was decided that the additional chaplain in fiscal year 1961 be research oriented and be employed as a research associate at the level of about GS-9, and to start making inquiries about possible candidates for the new position.

In April inquiries were sent to 22 supervisors of chaplaincy training programs and to 10 seminary professors of pastoral care for recommended candidates for the research associate position. It became apparent that persons well trained and qualified both in chaplaincy and in research methodology were unavailable. Research persons in the behavioral and social sciences and in pastoral psychology were consulted widely for recommendations regarding desirable qualifications and candidates for the consultant position. It became clear that, while valuable contributions could be made from several of the behavioral and social sciences and from pastoral psychology, no adequate research methods have yet been evolved for studying the spiritual needs and care of the sick. However, Dr. Dittes, whose research training is in the behavioral sciences, was highly regarded. The decision was made in July to confer with him about the position. He accepted in August.

Upon Dr. Dittes' advice, it was decided to up-grade the job description for the research associate to require a Ph.D. in the behavioral sciences and to make it a more permanent position. Further inquiries for candidates at this higher level of research competence, permanence and salary uncovered no candidates available for at least another year.

The following compromise was agreed upon:

1) To secure a research associate with adequate clinical training for chaplaincy work and with a Master's degree, one such possibility being available.

2) The new chaplain to undertake a two-year survey study of the nature and extent of the spiritual needs of the Protestant patients, with guidance by the research consultant as to sound methods.

This compromise plan would: (a) help provide needed service to patients; (b) give time to work out better understanding of our program with persons responsible for doctoral programs in this field; and, (c) give time to find a qualified chaplain with a Ph.D. for a more permanent position.

In October, Dr. Dittes interviewed two candidates and decided on the Rev. Robert I. White, who came well recommended for his ability as a chaplain. A job description was written and approved at the GS-9 level. Mr. White reported for duty 3 January 1961.

SUMMARY OF SERVICE - Protestant

1) Ministry to Patients

Initial interviews	1,306
Counseling	84
Rounds	1,338
Criticals	907
Baptisms	2
Funerals	3
Pre-Oper.	606

2) Worship

Chapel	54
Attendance	4,028
Average	77
Holy Communion	14
Chapel	685
Bedside	206

3) Patient Referrals

Doctors	63
Nurses	704
Social Workers	124
Clergy	41
Family	303
Patient	62
Others	33

4) Staff Relations

Counseling	28
Conferences	1,050
Staff Mtgs.	22
Lectures	21

5) Community

Addresses	10
Services	12
Meetings	38
Professional Conferences	22

6) Administration

Correspondence	968
Planning	340 hrs.
Conferences with Chaplains	73
Study & Research	760 hrs.

CATHOLIC CHAPLAIN

I. The religious needs of the Catholic patients at the Clinical Center are provided for by one Catholic Chaplain on full-time duty.

Services provided for by the Catholic Chaplain at the Clinical Center are summarized below in a general manner followed by a detailed summation of daily activities for the calendar year 1960.

The Catholic patient census continues to maintain an average of twenty percent of the total patient census, which amounts to an average of 75 to 80 patients. This number of patients requires the Catholic Chaplain to spend sixty hours a week to fulfill his duties properly.

Personnel continue to make demands on the Chaplain's time for personal appointments, but these demands must necessarily be subordinated to the needs of the patients. As a result, only about fifty percent of the personnel request are satisfied. The Catholic Chaplain continues to refer these personnel to their own clergymen in the community whenever possible.

During the past year the Catholic Chaplain, in conjunction with the Protestant and Jewish Chaplains, and at the suggestion of Dr. Himmelsbach, met with a research consultant to consider the possibility of doing some research projects regarding the religious needs of the patients. The Catholic Chaplain, while recognizing the basic value of such a project, felt that he understood the religious needs of his patients and therefore would not be benefited by participating in this project.

There is nothing else in the way of new projects of special interest to add to this report. The Catholic Chaplain's time is consumed in a normal routine of duties as summarized and detailed below.

II. GENERAL SUMMARY OF SERVICES PROVIDED BY THE CATHOLIC CHAPLAIN

1. Divine Worship Service (Mass) for patients, relatives and personnel daily. (Two services on all Sundays and Holydays).

2. Special religious services daily during months of May and October.

3. Special religious services during seasons of Advent and Lent.

4. Sacraments administered to patients daily.

5. All patients visited each Saturday for confession.

6. Seriously-ill patients visited every day.

7. Weekly consultations with families of patients.

8. All patients visited on regular schedule for counseling.

9. Consultations with Medical, Nursing and Social Service Staffs regarding welfare and rehabilitation of patients.

CATHOLIC CHAPLAIN - continued:

10. Participation in Orientation program of all new Nursing and Social Service personnel.

11. Participation in programs of Patient Activity Department.

12. Participation in Gray Lady Training Program.

13. Consultation with personnel on request.

14. On call for emergency service twenty-four hours a day.

CATHOLIC CHAPLAIN

III. DETAILED SUMMARY OF DAILY ACTIVITIES

I. CHAPEL SERVICES

Masses	371
Attendance	10,219
Sermons	52
Holy Communion	11,417
Chapel	4,313
Bedside	7,104
Devotions	48
Attendance	1,677

II. MINISTRY TO PATIENTS

Wards Visited	4,753
Initial interviews	565
Counseling	1,165
Seriously Ill	1,591 visits
Pre-Ops.	222 visits
Confessions	1,144
Chapel	109
Bedside	1,035
Baptisms	4
Confirmations	3
Last Rites	76
Funerals	3
Follow-ups	172

III. PATIENT REFERRALS

Doctors	396
Nurses	685
Social Workers	89
Clergy	119
Family	750
Patient	162
Others	171

IV. STAFF RELATIONS

Counseling	516
Conferences	6
Staff Meetings	12
Lectures	37

V. ADMINISTRATION

Correspondence	193
Planning	42 hrs.
Conference with Chaplains	6
Study & Research	119 hrs.

VI. COMMUNITY

Addresses	2
Meetings	0
Professional Conferences	1

JEWISH CHAPLAIN

Jewish chaplaincy service is on a contract basis providing for twelve visits per month. Rabbi Joseph M. Brandriss has been serving as Jewish Chaplain since November 24, 1956. Approximately 8% of the patients admitted to the Clinical Center are Jewish.

SUMMARY OF SERVICE

Sabbath services on Fridays and special service for the various major and minor Holidays of the Jewish calendar, including some socials after the celebrations.

Visits to patients.

Counseling of patients and families.

Religious education when requested and/or needed.

On call for emergency service twenty-four hours a day.

Calendar Year 1960

PUBLIC HEALTH SERVICE, NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER
ADMINISTRATIVE BRANCH

EMPLOYEE HEALTH SERVICE BRANCH

CC-12
Serial Number

Major Goals

The major goals of the Employee Health Service Branch of the Clinical Center remain unchanged and continue to be pursued under existing laws and regulations by providing occupational medical services to employees. In meeting its dual responsibility, the Employee Health Service first assists management in the prevention and control of occupational injuries and diseases, endeavoring to reduce sickness absenteeism and thereby improving working efficiency and productivity. In meeting its obligations to employees, it gives prompt and effective treatment for those occupational diseases and injuries which occur despite its best preventive efforts; and, in general, programs consistent with accepted preventive medicine health practices are developed and executed to assist employees in maintaining their health at the highest possible levels.

Major Progress

A comprehensive survey of the operation of the Employee Health Service was conducted during the year by a Clinical Center management team. Reported reactions by top management to the survey's findings and recommendations suggest that basic support for the overall program continues to exist. We believe that the survey will be a major factor in refocusing attention on our major goals, particularly in areas of preventive medicine. Future annual reports will tell the full story as we attempt to implement the survey's recommendations.

With the assignment of a Senior ranking Commissioned Officer to the Employee Health Service in the past year, it is hoped that the position of the Associate Medical Officer will be occupied for a longer period than in the past when two year Junior Officers were assigned.

Preventive medical services have occupied the primary interest of the staff during the past year. Immunization programs have been expanded and improved, and health counseling and referral procedures were strengthened. A comprehensive glaucoma detection program has been started during the year and is progressing satisfactorily. Detailed studies have been made on the feasibility of developing an effective rehabilitation program for problem drinking, and our preliminary findings offer considerable evidence for en-

couragement.

Major revisions have been made during the year in the methods of acquiring work-load statistics, and a major advance has been made in a continuing study of sickness absenteeism. Statistically valid methods have been developed to study the causes of sickness absenteeism in employees taking three to ten days of sick leave per pay period.

Lines of communication between various intra and extramural activities have been strengthened during the year, resulting in improved relationships and understanding with the NIH Safety Officer, NIH Fire Department, Bureau of Employees Compensation and the Public Health Service Out-Patient Clinic. We are looking forward to even better relationships in the future.

Relationships with NIH Clinicians have continued on a mutually beneficial basis, in that highly desirable clinical research material has been uncovered in the employee population with direct medical benefits resulting for the employees concerned. As in the past, in all non-emergency referrals of employees to the Clinical Center, the employee's private physician was consulted and requested to act as the referring physician for admission. As a result of this constant concern for ethical private physician-patient relationships, cooperation with private practitioners in the community has continued on the same high level which has existed in past years.

Major Problems

Overcrowded conditions arising from space deficiencies remain one of our greatest problems. A thorough study of space utilization and needs has been requested and we are hopeful, although not too encouraged, that some improvements will be possible. Staff shortages constitute the next major problem, with the need for an additional part-time physician and supportive nursing and clerical personnel being most urgently needed. The present level of effectiveness in our operations cannot be maintained indefinitely with our present staff in view of the increasing demands placed upon us.

A continuing problem, although one that is being gradually minimized, is the misunderstanding and relatively poor acceptance of the Employee Health program and services available. As greater efforts are made to expand our preventive medical services, we are hopeful that more employees will understand the basic goals of the program and realize that curative medical care cannot be, and never has been intended to be, a part of the Employee Health Program.

Selected Statistics

	FY 1960	1959	1958	1957
Total Visits.....	32,589	27,579	28,014	28,646
Visits for Occupational Injuries and Diseases.....	4,904	3,138	2,600	2,986
Immunizations.....	12,411*	15,604	13,154	8,760
Pre-emp. Physical Examinations.....	1,937	1,704	1,828	1,637
Laboratory Examinations.....	16,864	12,332	12,868	6,528
Referrals to Personal Physicians.....	2,024	1,736	1,631	1,454

*1959 report included 3829 smallpox immunizations given during an intensive campaign.

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH
SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
THE CLINICAL CENTER
SOCIAL SERVICE DEPARTMENT

SERIAL NUMBER CC-21
CALENDAR YEAR 1960

As the reports of the Social Service Sections of this Department are reviewed for the data for this overall report, one is struck by the evidence of maturity, of greater clarity of purpose, of the degree of acceptance and utilization of social work by the various other professions concerned with patient care and research, and by the widespread recognition this Department and its program have received as evidenced by the number of visits, requests for consultation and the times when our advice has been sought regarding ways in which social work and social research programs in other institutions should be organized. The plans, staff and goals of eight years ago have crystalized and taken form. A staff of twenty-eight professional clinical social workers is on duty. Seven of them have completed training beyond the Master's degree; two have acquired Ph.D.'s, and four are preparing doctoral dissertations directed toward that academic goal. The belief that social workers have a contribution to make in clinical research has been justified and the fact that social caseworkers can be taught to be effective in research techniques and methodology, which had been questioned by some members of the profession, has been demonstrated. Evidence of this is detailed in a later section of this report. The need for and value of the use of group work techniques in a hospital setting have been endorsed by medical and nursing colleagues.

The value and even necessity for a fund to provide for special needs of patients and their family members which cannot be met by appropriated funds has been proven. The generous support of the National Institutes of Health Recreation and Welfare Association, supplemented by gifts and contributions from many other organizations and individuals, has not only provided for simple needs of patients but has, in many instances, been the deciding factor in maintaining morale so that patients have been enabled to remain here and contribute to research goals. This Fund has had a further value in meeting urgent financial distress of family members who could not otherwise remain near their sick relative during critical or terminal phases of their illnesses, and has protected the good community relations of this institution which might have been endangered if indigent non-residents were drawn here to be with their sick relatives and became a source of concern to local agencies.

PEDIATRIC PATIENTS

The growing number of pediatric patients is developing a new dimension of service for the Social Service Department. While nursing unit 2-E is the only Pediatric Unit, child patients are scattered throughout many other nursing units of the Clinical Center. On 2-E, the child with an incurable disease poses many problems of an emotional or psychological nature for the parents and the staff. The social worker on that unit has to deal with strong feelings of anxiety and guilt on the part of parents who feel

responsible for the child's illness. The inevitable terminal phase of illness on the part of these children also creates many psychological problems for staff members. An innovation this year of the addition of a pediatrician to the medical staff serving this nursing unit, and a part-time psychiatric consultant, has given a greater sense of security to the members of the staff, and has increased their understanding of their own reactions to the illness of these children.

Early in this calendar year, the Social Service Department accepted a Bennington College student for assignment to assist on this unit under the close supervision of the social worker. She planned the play activities for the children and recorded her observations of the childrens' reactions to personnel, routines, events and other people. Although the preparation of this student for this activity was time-consuming, it was the general impression of the social service worker that the experiment was worthwhile. Of particular note was the fact that the children were enthusiastic in their acceptance of a college-aged person in their midst.

The middle of September marked the termination of service of the worker who had been on this unit for five years, and another worker was assigned this responsibility. In making the change the situation was evaluated and many frustrating problems related to this service were highlighted. In this review it was apparent that children with terminal illness present a social worker with a serious dilemma, namely, that of working closely with each sick child and possibly facing the interaction and support needed by the child who has periods of pain and fear, and working closely with the parents in helping them to express their anxiety and therefore attempt to alleviate their grief and pain. It requires a high degree of skill for the worker to provide this support for both parents and child, and not to become overwhelmed by the aspects of terminal illness in young patients.

The fact that the cause of the nature of the illness and the age of the children necessitates at least one parent remaining with the child living in the area of the Clinical Center poses additional problems which will be discussed in a later section of this report.

Another approach to a different type of problem among child patients is that which has been undertaken with the children on the Arthritis and Metabolic Diseases nursing units. These children often are subjected to extensive metabolic studies, and are frequently bored by the routine as well as made quite anxious by painful procedures which are necessary for the research itself. They had been creating a series of disciplinary problems, and with the strong support of the members of the nursing staff we have continued to provide the services of one social worker who has extended a group work-casework program for these children. This worker has utilized his social work skills by organizing group and recreational activity toward helping individual members of the childrens' group with the emotional difficulties which hamper their adjustment in the hospital and mitigate against maximum use of hospital benefits. He was able to involve members of the nursing staff in the process of working towards therapeutic goals for the children. This has been accomplished through regular conferences, attendance at rounds, and informal conferences as needs arose. He has

emphasized the value of a whole cooperative effort on the part of all professional personnel involved. While he carried special responsibilities for the project, various members of the nursing unit staff have taken on responsibilities without which such a demonstration would not have been possible. The enthusiastic support of the chief nurse of NIAMD and her complete cooperation in all matters have contributed to the success of this program. Last year the project was mainly centered on the 9-W nursing unit, but during the course of this year it has been expanded to include the 9-E and 8-W nursing units. The Nursing Department has expressed interest and hope that such a program could be extended to other units, such as the Pediatric Unit on NCI and for child patients on NHI, but the limitation of staff time has not permitted this to be accomplished.

The activities undertaken by this worker do not replace those of a caseworker. He has had the responsibility to help the child within the hospital milieu in order to minimize the child's anxieties and to help nursing personnel with problems of management and discipline in general. The caseworkers assigned to the units have continued to carry on the usual casework services for children which also frequently involved the child's own and the caseworker's contacts with the families. For example, in the case of one child patient, a five-year-old boy hospitalized here since January of 1960, one worker carried casework responsibility and another carried the group work-recreational responsibility. This child's parents live over 250 miles away, and there was regular correspondence with them and personal contacts whenever possible at the time of their visits to the hospital. There has been more than the usual necessity for as many contacts as possible with the family, for the child was given a drug, (testeosterone), which produces premature puberty, with the result that he has undergone accelerated growth and other pubescent changes. Whenever a visit was anticipated from the parents, it was necessary to prepare them for the additional changes in the child's appearance to help them absorb the shock of seeing him. The group worker's activity with this patient was to help him understand that the startling changes occurring to him was the result of the medication he was receiving and something everybody knew about and understood, as well as to help him remain comfortable with his peers from whom he was different. It was the caseworker's responsibility to help the mother understand that she was creating serious problems within this child by constantly referring in correspondence to the activities of a younger brother and making our patient increasingly hostile towards the other little children on the unit. As soon as the mother had this brought to her attention she changed the tone of her letters so that the patient was not constantly reminded of his brother's enviable position at home and was reassured of his parents' love.

On the NIAID Social Service Unit there has been an increasing number of referrals of pediatric patients. These children may be referred for direct casework interviews, or the worker may be asked to help keep him in touch with his family by correspondence or by telephone. The great hope is to keep an isolated child in touch with his family and home activities. These children with their physical and emotional resources sapped by long illness have needed intensive help by the social worker as a part of an overall team plan in handling the feelings of apathy or anger that the depriving

experience of isolation may produce.

On the NHI, the Clinic of Surgery continues to admit a large number of children. In 1959, two hundred and forty-one children under sixteen years of age were admitted to this service. Generally, these children are here with their families. On the other hand, there are increasing instances of children admitted completely alone, such as children of Eskimo or Indian groups in Alaska. The quick moving pace of the Surgical Unit makes it necessary to work quickly, and especially urgent is the need when considering the preventive aspects of casework intervention. It has been the experience of the worker on that service that frequently children can verbalize their specific fears and fantasies and if these can be handled prior to surgery, anxiety is decreased. However, the child who is too compliant and uncommunicative, with an absence of affect, is one who is of most concern. The entire problem of how best to prepare children of different age levels for heart surgery is one area in which the caseworker is constantly seeking a solution. The effect of the parents' fears and anxieties upon the child, particularly those under five, are frequently dealt with by the worker and may be the major focus of casework treatment. The increased need for services for these children exists not only in the area of casework but in recreational and diversional activities. There has been an improvement in terms of the space available with the utilization of part of a solarium as a playroom. Because of the number of procedures, restrictions of movement, and rapid turnover, activities for these children are most difficult to schedule. However, there are many possibilities for the exploration of group play and possibly group therapy.

During this year, there has been an increasing number of children admitted to other research programs of the NHI, and this has implications for this Department in terms of working with the families, if they are available, and for working directly with the child who may not feel ill but is here for an extended period of time conforming to the research protocol.

In the other Institutes, where child patients are admitted, there are the complex problems of dealing with these children's needs in settings which are also housing adults or even aged patients. Long-term hospitalization for some of these children develops many needs, such as need for recreational services or diversional activities as well as educational services, etc.

The Social Service Department staff members are constantly seeking more effective ways to deal with the problems of child patients in a research setting. The worker on the Heart Surgical Service has written material for a pamphlet at the request of Dr. Morrow, to inform patients what to expect while they are here for diagnosis and/or surgery.

SOCIAL PROBLEMS OF SURGICAL PATIENTS

The Surgery Service of NCI has continued to need a great deal of Social Service help due to a variety of factors including: 1) The mutilation aspects of much of this surgery. 2) The proportionately large number of referrals for nursing home care following discharge. 3) The amount of work involved in rendering assistance in filing for various forms of social

security pension benefits for the older group who will be incapacitated, without funds and unable to work. 4) Financial assistance for medically indigent families who have often spent most of their money in previous hospitalizations prior to coming to the Clinical Center and, 5) appropriate referral for vocational rehabilitation or job placement where indicated.

The worker assigned to this service, until the first of November, did several short-term studies which indicated that during one week there was a 38% turnover of patients and 62% continued throughout the week on this service. In another week, it was observed that out of eighteen head and neck surgery patients, ten were recipients of public assistance. These factors make it necessary for the worker to be fully knowledgeable about all community resources, not only within the area of Metropolitan Washington but also throughout the country. However, in addition to tangible environmental services, a larger percentage of casework services are rendered in the area of helping the patient and/or the family with their anxiety over the diagnosis, recurrence of illness, diagnostic waiting period, and with recommendations where patients cannot be hospitalized because they are unable to fit into a treatment protocol and arrangements must be made for their return home. Financial assistance is given when necessary to maintain patients' morale in keeping a family in the area during the period of surgery and the post-operative period. In this connection the Patients' Welfare Fund has served a very useful purpose.

On the Surgical Service of the NHI, there is a rapid turnover and the numbers of patients and their families facing this stressful period raise many problems for the caseworker. The social interaction of the family members themselves, particularly when there has been a death on the unit, is an ever impinging factor. One of the challenges of working with these patients is the fact that there are many who make a satisfactory social adjustment following surgery without physical improvement, and those with physical improvement but who, for one reason or another, are still unable to function more effectively.

Late in the year an administrative change was made on the NHI with the organization of a new Cardiology Branch and the Clinic of Surgery's division into two surgical services. This emphasizes a previous request for an additional worker to be assigned to the Heart Institute, and should such a request be approved budgetarily, will offer the opportunity to provide additional service where it is greatly needed.

On the NINDB, the Neurosurgery Unit has as its major medical emphasis the temporal lobe epilepsy study for which the worker does at least a social evaluation on every patient presented as a possible candidate for surgery. Continued casework services to these and other patients are given as indicated. The use made of these social reviews in total planning for individual patients has again proved valuable as it has been demonstrated in the past. The addition of subsidiary medical projects on the neurosurgical service has been more noticeable in this past year; with the addition of three new ones there are now a total of eight medical research projects on this service. The obvious result is that the worker must be alert to and informed about an increasing number of research studies even though there is no direct

participation, such as the study of electrolyte metabolism in epilepsy. A fact worth noting is that the Involuntary Movements Project, although small, is requiring considerable social service time because of the patients' marked speech handicaps.

In a study done this year, this unit showed that 62% of these patients stayed a month or less. This is caused, we believe, by the fact that there had been fewer patients admitted on whom neurosurgery is performed. This means discharge of a greater number of patients immediately after the completion of diagnostic studies. We further believe these data reflect the influence of different supervising physicians on the Neurosurgical Unit. This means that it will not be possible to predict what the casework pressures will be until the worker knows how the supervising physician will work.

This section of our report has been included, both for its informational value and also to suggest possible avenues of social work participation as we move toward the opening of the new surgical wing which will increase the amount of surgery performed by these three Institutes.

SOCIAL WORK PARTICIPATION IN RESEARCH

With the passage of time, the evidence becomes more substantial of the social workers' role in research projects. This participation has ranged from facilitating research by the provision of casework services which may assist a patient in coming to the Clinical Center, and in remaining at the Clinical Center during the period of research, and in freeing of a research bed as rapidly as possible by facilitating the discharge of the patient, to the collaboration of research to the extent of project design, review of the literature, selection and screening of patients and their families, and in the preparation of reports related to the findings of research projects. As has been true in the past, the social workers assigned to the Mental Health Social Service Unit have been most actively engaged in collaborative research. The extent of their involvement has become so extensive that in several instances workers are not involved in actual casework services to patients. This was recognized, and their functions were defined by a new position in this Department, which we term Clinical Social Worker (Research). At the present time two workers are so classified. It is anticipated that additional workers may qualify for this classification in the future. Some examples of the type of involvement which the Social Service Department staff members have in the research activities of NIMH are given as follows: The worker assigned to the College Project shared responsibility for the screening and selecting of patients and was solely responsible for seeing the families for therapeutic purposes. Another worker assigned to a different phase of the college project, namely, studying the emotionally well-adjusted college students, is responsible for the formulation and development of the research design of the family study aspect of the overall research plan, project planning, data collection and data analysis. In addition, she has been asked to participate in a training course for a selected group of women to do psychiatric counseling of college students as part of the on-going research project. Three workers are assigned to the Bio-Social Growth Study Project. Two are concentrating on the Newly Married Couple Study and one on the Nursery School Project. The first two devote the majority of their time to screening the prospective

couples and incorporating them into the project. The other is responsible essentially for recruitment of children for the nursery school, and it is interesting to note that they devote 85% of their time to the project and the remaining 15% is devoted to Social Service Departmental activities.

The workers assigned to the NINDB Social Service Section have been involved in a series of projects. For example, two of the individuals on that unit have a series of forty cases in which structured interviews have been held with patients to evaluate changes in social functioning of epileptic patients following neurosurgery, and are now in the process of evaluating their data. Another worker on this unit has been collecting data related to the social effects of eye disease over a three year period, and has been moving toward the completion of a study with regard to occupational changes experienced by patients, and their expressed feelings about eye disease as they relate to such variables as length and degree of vision lost. Still another worker has been undertaking a survey of the social effects of myasthenia gravis.

On NIAMD, one worker, in cooperation with a member of the Nursing Department and a psychiatrist, has participated in a cooperative study of emotional stress in the metabolic studies unit, and this material has progressed to a point where the preliminary findings will soon be published.

On NCI, the worker assigned to the Pediatric Unit has been involved in the development of a study of stress which will be an interdisciplinary project in which mothers of children with leukemia will be studied to determine the effect of emotional stress they may experience during the course of the progress of their child's disease. The entire group of staff on the Cancer Social Service Section have been undertaking a study of factors related to discharge planning, and the program supervisor has initiated a study of the psycho-social factors in dermatology patients.

STAFF DEVELOPMENT

Staff Project on Role Persistence:

Parsons'[1] studies and those of other sociologists[2] indicate that the person who is hospitalized must assume a new role--that of being a patient. There has been considerably less focus; in fact, to our knowledge, no focus on the patient's attempt to maintain himself as a functioning member of the family unit during the long-term hospitalization experience. For example, the effort of young mothers to continue to supervise the children's school and recreational activities by phone and letter. The father's interest in carrying on the financial planning for his family; and the young wife's

---

[1] Parsons, Talcott, The Social System, Free Press, Glencoe, Ill., 1951, Esp. p. 64.

[2] Simmons, Leo W., "A Frame of Reference for Family Research in Problems of Medical Care," Research in Public Health, New York, N. Y., The Milbank Memorial Fund, 1952.

attempt to supervise marketing and meal planning for her husband. The need on the part of the elderly patient to continue to provide emotional support to his wife who has grown to rely on him exclusively. The success or failure of these efforts, carried on under difficulties created by physical incapacity, distance and length of stay, frequently affect both participation in research and the decision to remain for the duration of the research project.

These efforts on the part of the patients were systematically observed by the social service staff during the exploratory phase of the staff project. Subsequently, the total staff at a general meeting affirmed this observation out of their clinical experience. It is on the basis of these observations from systematic exploration and clinical experience that the major hypothesis for the staff project has been formulated and set forth to be tested. It is as follows:

> The adult patient who is hospitalized thirty days or over and is a member of a nuclear family tends to attempt to maintain in the hospital whatever role and/or roles in the family he perceives to be his significant contribution to the welfare of the family unit.

In the light of social work's interest in total family functioning during the hospitalization experience, it seems particularly important and appropriate for us to begin to identify and study this phenomenon. It is additionally important to consider this phenomenon in the case of the chronically ill patient for whom long-term hospitalization is one point on a continuum of adjustments he makes in relation to his function in the family unit. The more traditional psycho-social history obtained upon hospitalization or upon referral during hospitalization provides more social facts, more longitudinal and attitudinal data than it provides a systematic focus on the patient's uniquely, crucial function in the family and the dispositions of this function at the time of hospitalization.

If our study indicates that this phenomenon does exist in a sufficient number of cases, it can provide at the point of admission and/or referral, a "new and, possibly, more accurate pair of glasses" with which to assess and predict more quickly and validly the import of the long-term hospitalization experience for the patient as a member of the family unit.

If we are clear early in the patient's stay about what he considers his crucial function in the family, we can work with him toward carrying out the task in the most comfortable way in terms of his physical conditions and the medical research problem or we may work with the patient toward modifying his task in order to fit in with his responsibilities as a patient. This approach reinforces his worth as a contributing member of the family and provides for his optimum participation in the research project.

During 1960, the exploratory data was reviewed and analyzed; the major and subsidiary hypotheses were formulated; the questionnaire was constructed and a workshop conducted on "Questionnaire Construction". At the present time, the pre-test of the questionnaire is being given to a few patients who have been at the Clinical Center thirty days or longer and are members of

nuclear families. It is anticipated that full scale interviewing for the project will be carried on during March and April with each staff member being responsible for four research interviews. This will provide the one hundred cases needed for the study.

Staff Project on Loneliness of Family Members:

In the exploratory interviews for the staff project, the problem of loneliness was brought out by a majority of the families of the patients. Because there was awareness on the part of the staff that neither social work records nor social work literature reflect this important concern for the family, the idea was referred to two staff members for further exploration preparatory to a more extensive study on the part of the whole staff.

Thus far, the staff members on the project have completed fifteen exploratory interviews. Through this exploration and a series of conferences, the entity of loneliness has been described for the purposes of a social work study; the exploratory interview has been focused in an increasingly systematic approach; certain techniques for eliciting material in this sensitive area have been devised; and preliminary observations have been detailed.

It is the present impression of the two staff researchers, that families tend to handle loneliness by increased activity (busyness); visualizing or imagining the patient still at home; and attempting to increase communication with the patient. They appear somewhat relieved by reassurance that the patient is receiving good medical care; and an opportunity to discuss their feelings of loneliness.

The question remains whether or not the family member's loneliness is ever really reduced more than temporarily; and how this continued emotional state affects the social functioning of the family member.

It is anticipated that interviewing and discussion around the entity will be continued in order to describe the phenomenon adequately. Ultimately, we wish to use this knowledge toward a better understanding and handling of the family's feelings of loneliness by the social worker.

It is of note that a report of the preliminary observations in this study was of particular interest to the Scientific Director of the Heart Institute.

Staff Project on Assessing Strength in Dealing with the Problems of Long-Term Hospitalization:

The exploratory phase of the staff project suggested differing capacities on the part of the patients and families in dealing with the difficult problems occasioned by long-term hospitalization as a result of chronic illness.

It was deemed important to begin to assess those capacities--social and emotional--which enable a patient and his family to cope more adequately with these problems. A systematic understanding of those capacities which

make for strength and resiliency will enable us to reinforce them where present and help provide them when it is not a part of the patient and family's natural equipment.

The preliminary project to set forth criteria for assessing strength in social functioning is being conducted by two staff members.

Workshop on Questionnaire Construction:

To further the Department's objective of helping each worker to be oriented to the research process in preparation for participation in multi-disciplinary research and social work research, the program of staff workshops in relation to each phase of the staff project has been continued.

In the early fall, Dr. Lelia Deasy, Associate Professor of Social Work Research, Catholic University, and formerly a staff member of the Laboratory of Socio-Environmental Studies, NIMH, led a workshop on questionnaire construction. The questionnaire devised for the staff project was used as a basis for a formal lecture and a discussion session.

THE CASEWORK WORKSHOP

The Casework Workshop for 1960, is being conducted by the Program Supervisor, NHI. It is designed to provide an opportunity for staff members further to refine their diagnostic and treatment skills in dealing with casework problems in a medical research center. The emphasis is upon an early and accurate evaluation of the patient and family's ability to handle the hospitalization experience; and the appropriate choice of social work treatment.

The cases chosen for discussion range from medical problems with a major emotional component to problems of young parents facing the imminent death of a child, to overwhelming anxiety in contemplating surgery.

Projected Plans:

During this year, staff members have taken increased responsibility for individual research projects, and for leadership in discussion groups. It is our objective to foster this kind of creative contribution to staff development, and ultimately to social work knowledge through additional opportunities for participation in individual projects; and for guidance through planned discussion groups.

PROBLEMS

1. The extensive use of the Patients' Welfare Fund to support family members of patients facing surgery or critically ill should be noted. It can be expected that this need will increase.

2. There is a growing need, as previously reported, to establish a better system for obtaining interpreters. Such persons are needed for more sophisticated purposes than simple translating. Non-English speaking patients need to be reassured, to be given emotional support in times of crisis, to have their physical and emotional needs conveyed to staff, and need detailed knowledge of their anticipated medical and/or surgical procedures which can be given them only by a knowledgeable person. Need for interpreters should be recognized when pre-admission actions are underway.

3. Space for privacy in interviewing remains a problem. At this point, two persons share every office assigned to the Department. No resolution for this problem can be seen in the immediate future.

4. With the number of clinical social workers on duty and because of the volume of casework recording, pressure is mounting for a small pool of three dictating machine transcribers to be assigned to the Department to relieve the secretarial staff of this duty.

5. The change in policy related to the Welfare and Retirement Fund which eliminates its use for miners who have been unemployed for a year (and their dependents) is throwing an additional burden on the Patients' Welfare Fund. For example, $586.06 was paid out of the United Mine Workers' Fund in July, the month the policy went into effect, and only $158.66 in December.

6. The interesting demonstration of the value of a group work program in a hospital setting which has been so successful on NIAMD should be extended. This can only be done by budgeting for additional group workers in the Social Service Department. This would seem to be a worthwhile investment and very much needed and wanted.

This year it is impressive to see the evidence of a number of papers published or presented for publication in journals of prestige.

1. "Preventing Dependency Patterns in Chronically Ill Children", Jessie P. Dowling, Journal of Social Casework, October 1960.

2. "The Emotional Problems of Children Facing Heart Surgery", Roberta Peay, Children, November-December 1960.

3. "Distressful States Experienced by Patients in the Clinical Center", Paul Blank, Shirley Owen and Roberta Peay. To be published in Hospitals.

4. "The Sick Patient and his 'Well' Sibling", Stanley I. Hirsch, Co-Author. Presented at American Psychiatric Association and prepared for publication.

5. "Social-Psychological Characteristics of Old Age", Paul Blank, Co-Author. To be included in Monograph on Aging.

6. "Characteristics of Parents in a Field Group Study", Elizabeth B. Murphey, Co-Author.

7. "A Study of Distortions in the Recording of Caseworker's Interviews", Charlotte Wilkie.

8. "Hospital Care of the Chronically Ill Child: A New Approach", Joel Vernick, Co-Author.

PROFESSIONAL ACTIVITIES

Dr. Daniel E. O'Keefe

Chairman, Psychiatric Social Work Section
National Association of Social Workers
July 1, 1959 - June 30, 1961

Member, Board of Directors
National Association of Social Workers
July 1, 1959 - June 30, 1961

Member, Medical Social Work Advisory Committee
American Cancer Society

Miss Ellen J. Walsh

Member, National Practice Committee
Medical Social Work Section
National Association of Social Workers
1959 - 1961

Lecturer, "The Meaning of Illness"
American National Red Cross Training Center
March and October 1960

Member, Public Relations Committee
Washington Chapter
National Association of Social Workers

Teacher, Seminar in Supervision
Catholic University
October 1960 - January 1961

Mrs. Jessie P. Dowling

Chairman, Washington Chapter
National Association of Social Workers

Member, Delegate Assembly
National Association of Social Workers

Mrs. Kathryn K. Kilian

Member, Intake Committee
Health and Welfare Council
Washington Metropolitan Area

Professional Activities (Continued)

Mrs. Kathryn K. Kilian and Miss Mary Ann Fugitt

Members, Social Work Sub-Committee of the
Professional Education Association of the
D.C. Division of the
American Cancer Society

Mr. Lawrence Burke

Member, Public Relations Committee
Washington Chapter
National Association of Social Workers

Miss Rintha Carter

Member, Personnel Practices Committee
Washington Chapter
National Association of Social Workers

Miss Roberta Peay

Reprint Committee
Medical Social Work Section
National Association of Social Workers

Program Committee
Medical Social Work Section
Washington Chapter
National Association of Social Workers

Mr. Delbert Nye

Chairman, Welfare Committee of R & W Association

Miss Joan Lindall

Recruitment Committee
National Association of Social Workers

Mrs. Charlotte Wilkie

Chairman, Psychiatric Social Work Section
Washington Chapter
National Association of Social Workers

Member, Executive Committee
Washington Chapter
National Association of Social Workers

Professional Activities (Continued)

Mrs. Charlotte Wilkie (Continued)

Member, Education Committee
Washington Chapter
National Association of Social Workers

Member, Education Committee
Washington Chapter
National Association of Social Workers

Member, Health and Welfare Council Steering Committee
on Mental Health Resources

Member, Board of Directors of Montgomery County
Mental Health Association

Chairman, Speaker's Bureau of Montgomery County
Mental Health Association

Delegate of Montgomery County Mental Health Association
to Maryland State Conference on Mental Health

Social Worker Consultant to NIH Employee's Health Service

Mr. Robert Mahon

D. C. Medical Society
Committee on Rehabilitation

Director of Montgomery Center for
Discharged Mental Patients

Member of Rehabilitation Committee
Montgomery County Mental Health Association

Mrs. Carmen Cabrera

Member, Personnel and Practice Committee
Washington Chapter
National Association of Social Workers

Chairman, Planning Committee
HWC D. C. Area

Resource Personnel Panel, Puerto Rico Delegation
White House Conference

Consultant, Group for Review and Development Community Resources
District of Columbia

					624			
					398	966		
				33	487	392	157	
				17	354	366	145	
					467	374		
					397			
					496	385		
					668	380		
					583	356		
					460	288		
					500			

	10	14	5	1	0		
A pril	9	15	6	1	0		
S eptember	22	1	10	1	4		
O ctober	16	12	10	1	17		
N ovember	14	6	9		15		
D ecember	23	8	9		16		
J anuary	22	9	13		16		
F ebruary	19	5	12		16		
March	26	12	16	1	15		
A pril	21	11	10		17		
May	21	14	16		16		
June	17	10	11		13		
Total	254	116	135	5	181		

	Patient Medicine, Drug Indian, O.T., etc.	Patient Transportation	Allowances to Patients	Patient Misc. Expenditures, Telephone Clothes, Meals, Special Devices, etc.	Meals Recreation	Total Withdrawn
	3.76	55.60	140.60	82.69	368.35	694.80
		113.27	168.82	59.07	853.90	606.06
	10.40	141.65	277.30	58.00	252.90	740.20
	5.00	17.50	296.00	37.93	487.37	843.80
	9.10	18.90	134.85	35.50	217.30	405.05
		82.71	276.00	93.98	225.75	707.84
	45.16	59.30	603.66	27.00	634.60	1570.72
		138.99	335.00	13.50	379.99	867.48
	65.60	211.70	1138.50	38.67	486.19	1094.66
	19.05	77.00	679.75	201.31	563.62	1541.25
	24.65	127.16	509.80	144.97	387.72	1196.30
	75.65	44.00	457.00	291.14	437.65	1254.61
	$264.04	$1086.93	$5050.71	$1018.80	$5305.31	$12952.07

RECAPITULATION:

Patient Trust Funds Turned 7/2/59	$ 1,643.27
Patient Trust Funds 1960 Deposits	15,708.60
	17,351.87
Patient Trust Funds 1960 Withdrawals	11,984.36
Balance on hand 6/30/60	5,367.51

* Of the total amount withdrawn, $ 2,247.87 was used for the patients' welfare.

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT
CLINICAL CENTER

CC-34
Serial No.

ANESTHESIOLOGY DEPARTMENT

The Anesthesiology Department was concerned with its primary objectives of providing anesthesia and related supportive care for patients undergoing operative surgery, diagnostic procedures requiring general anesthesia, and for the administration of oxygen and other forms of inhalation therapy for all Clinical Center patients. Research activities included clinical projects, in which new anesthetic agents and methods were evaluated, collaborative work with investigators of NHI, NINDB, and NIDR, and the origination of a number of laboratory investigations which had a direct bearing on clinical problems encountered.

The full time staff consisted of the following personnel: (based on the yearly average)

Clinical Anesthesiologists	6
*Research Anesthesiologists	0
Nurse Anesthetists	3
Inhalation Therapists	3
Secretary	1
**Anesthesia Technicians	2

The services of two outside consultant anesthesiologists were utilized regularly to provide an additional anesthesiologist three days per week, on the days of the heaviest surgical schedules.

I. ANESTHESIOLOGICAL PROCEDURES. Anesthesia and supportive therapy was conducted in a total of 1,226 instances, for 844 surgical operations and 382 diagnostic procedures. In addition, diagnostic nerve blocks, consultations for respiratory and pain problems and emergency resuscitation measures performed outside of surgical areas, totaled 66.

A large proportion of the relatively complex surgical operations and diagnostic procedures performed at the Clinical Center required

* One visiting scientist (NINDB) was available for part time clinical work and rotated on emergency call.
** Nursing assistants assigned from the Surgical Nursing Service.

the maintenance of anesthesia over unusually long periods of time, for example:

Anesthesia time exceeded 3 hours in 483 operations (39%)
Anesthesia time exceeded 5 hours in 202 operations (16%)
Anesthesia time exceeded 6 hours in 97 operations (8%)

Surgical operations were classified as follows according to the type of surgery and the special adjunctive techniques were employed as indicated:

1.	Thoracic (cardiac, pulmonary and great vessels)	
	a. extracorporeal circulation technique	124
	b. *hypothermia technique	0
	c. usual technique	105
2.	Neurosurgical (brain and spinal cord)	
	a. hypothermia technique	19
	b. usual technique	107
3.	General surgical (abdominal, pelvic, and inguinal)	
	a. hypothermia technique	2
	b. usual technique	166
4.	Perineal (genito-urinary, gynecologic)	35
5.	Head, neck, and face	69
6.	Eye, ear, nose, and throat	19
7.	Dental (majority with detailed physiological monitoring)	116
8.	Orthopedic	26
9.	Miscellaneous procedures	56
10.	Total	844

From the above tabulation, it can be seen that most of the surgery performed at the Clinical Center is concerned with such procedures as radical excision surgery for treatment of cancer of the pelvic organs and structures of the face and neck, cardiac surgery for correction of congenital and acquired defects with the aid of a pump oxygenator, so that the heart and lungs are bypassed during the period of definitive surgery, and surgery on the brain consisting of localization and removal of irritative foci as well as

*Local cooling of heart and of the blood in pump oxygenator done in 4 instances.

the removal of intercranial tumors with the aid of hypothermia. Operations entailing the use of extracorporeal circulation were carried out in 124 instances, whereas hypothermia techniques were employed in 21 operations, two of these patients having extensive liver surgery. At the end of the year, local hypothermia of the heart combined with cooling of the blood delivered by the pump oxygenator was combined with extracorporeal circulation as noted in the footnote, page 2. The preponderance of lengthy complex surgical procedures required the attendance of two professional anesthetists, and the services of trained anesthesia technicians were necessary for the operation of monitoring equipment and thermal devices. A surprisingly large number (116) of short duration dental procedures where elaborate physiological monitoring was carried out also required two anesthetists and one technician for their conduct.

Diagnostic studies done under anesthesia numbered 382. Many of the patients were small children who were unable to cooperate under local anesthesia. Methods of controlled sedation over periods of several hours were required for cardiac catheterization studies. Other tests done on adults required general anesthesia and complete muscular paralysis and respiratory control during selected periods. Since many of these patients were ill and the manipulations during the tests were capable of causing serious cardiac or respiratory reactions, special care was required in the anesthesiological management. Diagnostic tests done under general anesthesia are tabulated below:

Pneumoencephalograms	27
Cerebral angiography	95
Special eye or ENT examinations	12
Cardiac catheterization procedures	190
right heart catheterizations	64
trans-septal or percutaneous left heart catheterization	33
right heart catheterizations and angiography	44
angiocardiography or aortography	49
Cystoscopy	33
Miscellaneous	25

The following table indicates the institutes responsible for surgical cases with whom we worked. The first four institutes listed have surgical services. Patients sponsored by the last three

institutes usually have their surgery performed by surgeons of the institutes with surgical programs.

Sponsoring Institute	Procedures	Percent
* NHI	460	37
NCI	270	22
**NINDB	290	24
NIDR	110	9
NIAMD	50	4
NIAID	42	4
NIMH	4	0.3

II. INHALATION THERAPY SECTION ACTIVITIES. Inhalation therapy treatments were carried out by or under the direction of inhalation therapists. Consultation service was provided by an assigned anesthesiologist. The numbers and types of treatment during 1960 were as follows:

Oxygen tent therapy	5,393 hours
High humidity tent therapy	14,322 hours
Incubator with high humidity	4,436 hours
Hydrojette (cold steam)	12,544 hours
Oxygen administration by catheter mask	4,345 hours
Aerosol therapy	9,548 treatments
Intermittent positive pressure breathing with aerosols	1,067 treatments
Respirator use, long term	600 hours
Resuscitators (artificial or assisted respiration)	617 hours
Administration of oxygen combined with other gases	77 treatments

Orientation programs for new employees of the Nursing Department were carried out by means of weekly demonstration sessions. A total of 183 nursing personnel attended these sessions. The Inhalation Therapy Section sponsored a meeting of inhalation therapists in this area for the purpose of demonstrating techniques used in the care of patients who had undergone open heart surgery.

* Includes 190 diagnostic procedures
** Includes 134 diagnostic procedures

III. RESEARCH ACTIVITIES. The following clinical studies were carried out during the year. Individual project reports are attached for those projects in which members of our staff were the principal investigators.

1. The long-term project on the study of physiological changes occurring during general anesthesia for dental surgery on ambulatory patients, which has been sponsored by NIDR for the past three years, was utilized in the study of anesthetic methods in a search for techniques which would produce minimal physiological changes. Project report is attached.

2. Studies on the effect of various drugs on myocardial contractility and peripheral resistance were carried out on patients undergoing open heart surgery with the aid of cardiopulmonary bypass. Two separate projects emanated from these studies.

a. Measurement of the effects of a number of anesthetic agents and muscle relaxants was done by two of our staff in collaboration with two investigators from NHI. The project report is being submitted by NHI.

b. The effect of halothane on myocardial contractile force and peripheral resistance during cardiopulmonary bypass was the subject of a study by one of our staff serving, as principal investigator, and a colleague from the Surgery Clinic, NHI. The project report is attached.

Anesthesiology Department staff members conducted or participated in the following laboratory projects:

1. Animal studies were carried out in an attempt to elucidate the increased incidence of paradoxical hypertension which had been observed in patients following resection of coarctation of the aorta under halothane anesthesia. Project report is attached.

2. Studies were initiated on primate animals to ascertain the effects of muscle relaxants on cardiovascular functions. This work was done in collaboration with the research anesthesiologist of NINDB. The project report is attached.

3. Animal studies were begun to measure the effect of digitalization on the minimal effective dose of barbiturate required to produce a negative inotropic effect on the heart. This work was done in collaboration with an investigator of the Laboratory of Chemical Pharmacology, NHI. The project report is attached. A similar report has been submitted by NHI.

4. The relationship of myocardial catecholamines in the determination of the cardiovascular responses to anesthetic agents was begun in a project undertaken by one of our staff and a colleague from the Cardiology Branch, NHI. Project report is attached.

All the research activities enumerated above were done in collaboration with investigators from NHI and NINDB with the laboratory facilities supplied by these institutes.

IV. MAJOR PROBLEMS ENCOUNTERED. Personnel recruitment was not a major problem during 1960, although a great deal of time and effort was expended in arranging appointments two years in advance for physicians who desired to undertake residency training under the CORD program preliminary to fulfillment of their military service obligations at NIH. Two vacancies for inhalation therapy trainees were filled after overcoming the customary difficulties in obtaining Civil Service appointments for applicants with good potentialities but limited experience in the field. The increasing demands placed on the Inhalation Therapy Section made it imperative that we have trainees to take the place of inhalation therapists who leave NIH to enroll in college.

The major problem encountered during 1960 was the handicaps faced by our staff members who are interested in carrying out research work. In the original organizational planning of this Clinical Center Department, no direct provisions were made for staff anesthesiologists to engage in laboratory investigations. All projects involving laboratory work must be done under the auspices of one of the Institutes. As in past years, we were very fortunate in being permitted to collaborate with the investigators of NHI and NINDB in carrying out laboratory work. With a full complement of trained anesthesiologists on hand, and the tendency for our surgical schedules to peak during certain days of the week and certain periods of the year, we considered it to be essential that our staff be provided with full opportunities for research. The allocation of specific laboratory facilities would permit us to attract and retain one or more anesthesiologist with research interests as a permanent staff member. Such an arrangement could be expected to be of benefit in attracting permanent staff anesthesiologists in the future, when, and if, changes in the Doctors' Draft Act cut us off from our supply of newly trained specialists who have military service obligations.

V. PUBLICATIONS AND PRESENTATIONS.

The names of Anesthesiology Department Staff authors are underlined, the names of investigators from specific institutes are indicated, in the following summary:

1. Hebert, C. L.
   Physiological Monitoring of Patients During Anesthesia and Surgery.
   Presented at 13th Annual Conference on Electrical Techniques in Medicine and Biology, Washington, D. C., November 1, 1960.
   Published in Digest of Technical Papers

2. Bloodwell, R. D. (NHI), Brown, R. C., Christenson, G. R., and Goldberg, L. I. (NHI)
   The Effect of Anesthetic Agents on Myocardial Contraction in Man.
   Presented at 34th Congress of the International Anesthesia Research Society. Washington, D. C., April 5, 1960.
   Accepted for publication in ANESTHESIA AND ANALGESIA

3. Christenson, G. R., Hebert, C. L., and Driscoll, E. J. (NIDR)
   Intravenous Barbiturate Anesthesia for Dental Operations
   Presented at 34th Congress of International Anesthesia Research Society. Washington, D. C., April 5, 1960.

4. Hebert, C. L., Christenson, G. R. and Clark, M. A.
   Controlled Anesthesia and Anesthesia for Children Undergoing Cardiac Catheterization Procedures.
   Presented at Second World Congress of Anesthesiologists, Toronto, September 5, 1960.

5. Davis, T. B., Morrow, D. H., Cooper, T. (NHI) and Hebert, C. L.
   An Increased Incidence of Paradoxical Hypertension Following Resection of Coarctation of the Aorta under Halothane Anesthesia.
   Presented at Annual Meeting of American Society of Anesthesiologists. New York, October 3, 1960.
   Accepted for publication in ANESTHESIOLOGY.

6. Hebert, C. L. and Davis, T. B.
   Workshop on operating monitoring techniques.
   Presented for Southern Society of Anesthesiologists at Clinical Center, April 29, 1960.

7. Goldberg, A. H., Maling, H. M. (NHI) and Gaffney, T. E. (NHI)
   Effect of Digoxin Pretreatment on Heart Contractile Force During Thiopental Infusion.
   Scheduled for presentation at the Annual Meetings of the Federation of American Societies for Experimental Biology, April 1961.
   Accepted for publication in Federation Proceedings

Serial No. CC-34
1. Anesthesiology Dept., CC
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Importance of Myocardial Catecholamines in Determining the Cardiovascular Responses to Anesthetic Agents

Principal Investigator: Dean Morrow, M.D.

Other Investigators: John Holman, D.V.M.
Thomas E. Gaffney, M.D.

Cooperating Units: Laboratory of Kidney and Electrolyte Metabolism and Cardiology Branch, National Heart Institute

Man Years: 4 days (project began late in December 1960)

Project Description:

Endogenous catecholamines are known to modify responses to anesthetic agents. Ether is reported to have a negative inotropic effect which is mitigated by simultaneous catecholamine release. Catecholamine depletion produced by reserpine is reported to be associated with hypotension during anesthesia.

Thus far, it has been impossible to study the role of myocardial catecholamines alone, in the response of the circulation to common anesthetic agents. Reserpine can be used to produce myocardial catecholamine depletion but it also depletes other tissues of catecholamines and interferes with adrenergic reflexes. Heart-lung preparations made from dogs pretreated with reserpine would provide a test situation with the advantage of catecholamine depletion but with the disadvantage of being an isolated, highly artificial, deteriorating preparation.

The availability of a new surgical technique that produces an areflexic, catecholamine depleted heart, in an otherwise healthy, intact dog, provides a unique opportunity to study the direct cardiac effects of anesthetic agents without the masking effects of protective reflexes and/or myocardial catecholamine release. In addition, because the animal is otherwise intact, the contribution of cardiac reflexes and myocardial catecholamine release to the systemic circulatory effects of anesthetic drugs may be evaluated.

The increased clinical use of therapeutic agents which produce cardiac amine depletion (reserpine, guanethidine) makes it necessary for the anesthesiologist to select agents which are the least harmful in the presence of amine depletion. This study will help determine this question.

A comparison of the effects of common anesthetic agents upon heart contractile force, rate and blood pressure in normal dogs and dogs with areflexic catecholamine depleted hearts is planned. Appropriate dose ranges for each agent would be explored. One hypothesis is that the minimal dose of each inhalation anesthetic agent required to produce a negative inotropic effect would be less in the amine depleted heart, that is to say, the dose effect curve would be shifted to the left. Chloralose will be used as basal anesthesia to provide a preparation without reflex or cardiac depression. Heart contractile force will be measured with a strain gauge arch.

Initial studies will be done with halothane and succinylcholine.

Part B not included

Serial No. CC-34
1. Anesthesiology Dept. CC
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Cardiovascular Effects of Succinylcholine in Rhesus Monkeys

Principal Investigators: Thomas B. Davis, M.D.
Anibal Galindo, M.D.

Cooperating Unit: Neurosurgical Laboratory, NINDB

Man Years (calendar year 1960): 8 days, professional, remainder of personnel supplied by NINDB

Project Description:

Studies are being made to determine the dose relationship of succinylcholine to myocardial irritability and blood pressure changes utilizing primate animals. Monitoring of various cardiovascular parameters and anesthesia depth, by means of electroencephalogram, are done. Electrical stimuli are then applied to the left ventricle until a threshold voltage has been determined. Succinylcholine in divided doses is administered and its effect on the alteration of the response of the myocardium to electrical stimuli observed. Other drugs that may modify the threshold of irritability will also be included in the study. Lidocaine is one such drug. Similar studies will be done with d-tubo curare.

This study has considerable significance in that muscle relaxants such as succinylcholine and d-tubo curare are known to produce serious cardiac arrhythmias in certain clinical situations.

Part B not included

Serial No. CC-34
1. Anesthesiology Dept., CC
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: General Anesthesia for Dental Outpatients

Principal Investigator: G. R. Christenson, M.D.

Other Investigators: E. J. Driscoll, D.D.S.
C. L. Hebert, M.D.

Cooperating Unit: National Institute of Dental Research Clinic

Man Years (calendar year 1960): Anesthesiologists, 2 for ½ days each week, 52 man days
Anesthesia Technician, 1 for ½ day each week, 26 man days
Remainder of personnel supplied by NIDR

Project Description:

Objectives:

This study was initiated to gather pertinent data concerning vital physiologic processes occurring during dental surgery performed under light general anesthesia and to obtain comparative information concerning the relative merits of available anesthetic agents and methods.

Methods employed:

Continuous monitoring of the electroencephalogram, electrocardiogram, arterial oxygen saturation, and respiration as well as the recording of blood pressure at minute intervals is done on these patients. Patients are chosen by the National Institute of Dental Research staff and consist of ambulatory patients needing full mouth extractions who are in good general health.

Major Findings:

Our findings reveal appreciable physiologic stress as evidenced by a considerable rise in pulse rate and blood pressure during the period of anesthesia and surgical stimuli. These changes are decreased by analgesic premedication and by the supplementation of the basic intravenous barbiturate anesthesia with nitrous oxide inhalation.

The new oxybarbiturate, Methohexital Sodium, has proven to be more potent and to have a more rapid recovery period than other barbiturates tested. The new antisiologogue, hexocyclium methyl sulfate, has proven to be more effective and predictable and to produce less objective and subjective side effects than other drugs tested.

Part B not included

Serial No. CC-34
1. Anesthesiology Dept., CC
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: The Effect of Halothane on Myocardial Contractile Force and Peripheral Resistance During Cardiopulmonary Bypass

Principal Investigator: Dean H. Morrow, M.D.

Other Investigator: Andrew G. Morrow, M.D.

Cooperating Unit: Clinic of Surgery, National Heart Institute

Man Years (calendar year 1960): Fifteen days, professional

Project Description:

Halothane was introduced as an anesthetic agent by Raventos, et.al. in 1956. Since that time it has become a well established fact that in clinically useful concentrations it is capable of producing profound systemic hypotension. The etiology of this hypotension has been variously attributed to direct myocardial depression, and, or a decrease in peripheral vascular resistance. Bloodwell, et. al., from this clinic, reported a study of the myocardial depressant effects of the drug as measured with the Walton-Brodie strain gauge arch. They concluded that arterial pressure did not necessarily follow the depression in myocardial activity.

Objectives:

This study was designed to expand upon their work by assessing the central and peripheral effects of the drug physiologically separated by cardiopulmonary bypass.

Methods Employed:

Six patients undergoing routine closure of atrial septal defects under extracorporeal circulation were studied. Anesthetic premedication and maintenance operative anesthesia was selected to be well within limits previously found to have little effect on myocardial contractile force and arterial blood pressure under the conditions of the experiment. Halothane in a fixed two percent concentration was introduced into the pump oxygenator unit for a five minute period. Myocardial contractility and peripheral blood pressures were continuously recorded. Alterations in contractile force as measured with the Walton-Brodie gauge were

interpreted as direct effects of the drug on the myocardium. Alterations in peripheral arterial pressure, in the presence of a constant cardiac output, were interpreted as alterations in peripheral arterial resistance.

Major Findings:

Halothane in the stated percentage was found to decrease myocardial contractile force. This decrease was nearly linear with time. Peripheral arterial resistance fell, the rate of fall being slower than that of contractile force for similar times of exposure. With cessation of drug administration each parameter returned toward control values. It was concluded that the hypotensive effects of halothane may be attributed to two distinct physiologic actions of the drug.

Part B not included

Scheduled for publication

Serial No. CC-34
1. Anesthesiology Dept., CC
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: An Increased Incidence of Paradoxical Hypertension Following Resection of Coarctation of the Aorta Under Halothane Anesthesia

Principal Investigator: Thomas B. Davis, M.D.

Other Investigators: Dean H. Morrow, M.D., Theodore Cooper, M.D., and Clarence L. Hebert, M.D.

Cooperating Unit: National Heart Institute, Section of Experimental Surgery

Man Years (Calendar year 1960): 32 days, professional, remainder of personnel supplied by NHI

Project Description:

Sealy and others have reported that paradoxical hypertension occurs in about 10% of patients who undergo surgical correction of aortic coarctation. This same incidence occurred in the National Heart Institute in the past few years. However, during this past year five consecutive patients that underwent surgical resection of aortic coarctations developed postoperative hypertension. The one obvious difference in the handling of these five patients was that all received a new anesthetic agent, halothane.

Objectives:

Because halothane is known to have profound cardiovascular effects we selected to study this agent in relationship to the postoperative hypertension seen in those patients that had repairs of aortic coarctation.

Methods Employed:

Five mongrel dogs were studied, each dog twice. On one occasion nitrous oxide thiopental was the anesthetic and on another occasion nitrous oxide halothane was employed. Continuous intra-arterial pressures were measured before, during, and after an acute total occlusion of the thoracic aorta. After the chest was closed, the anesthetic gases were discontinued and arterial pressures monitored for one hour post-anesthesia.

Major Findings:

The results showed a definite tendency for postoperative hypertension to occur in the five animals that received nitrous oxide halothane anesthesia. Whereas in the five animals that received nitrous oxide thiopental the average pressures at one hour post-anesthesia, post-occlusion did not exceed the average control pressure.

Part B not included

Serial No. CC-34
1. Anesthesiology Dept., CC
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title: Effect of Digoxin Pretreatment on Heart Contractile Force During Thiopental Infusion

Principal Investigator: A. H. Goldberg, M.D.

Other Investigators: H. M. Maling, Ph.D.
T. E. Gaffney, M.D.
Martha A. Williams

Cooperating Units: Laboratory of Chemical Pharmacology, NHI
Cardiology Branch, NHI

Man Years (calendar year 1960): 12 days, professional, remainder of personnel supplied by NHI

Project Description:

Objectives:

To determine whether digitalization would affect the inotropic response to thiopental in anesthetic as well as lethal doses.

Methods Employed:

A strain gauge arch is placed on the heart of the dog which has been anesthetized with chloralose. Thiopental is infused intravenously. Myocardial contractile force, arterial blood pressure, venous pressure and electrocardiograms are recorded before and during the infusion. A comparison is made of the results of this procedure in control and digitalized dogs.

Major Findings:

In 6 of 8 digitalized dogs and 2 of 8 controls, the thiopental infusion initially caused an abrupt increase in contractile force (+5 to 50%), arterial pressure, and heart rate. The mean percent changes in heart contractile force produced by thiopental after 15, 30, and 60 mg. Hg. were -9.5, -33, and +60.8% in controls and +8.2, -13.7, and 28.8% in the digitalized dogs. This study should interest those persons who advocate preoperative digitalization of patients. The outlined experiments will be completed.

Part B not included (To be published in Federation Proceedings, April 1961.

Calendar Year 1960

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

CC-35
Serial No.

DIAGNOSTIC X-RAY DEPARTMENT

Program Goals:

The chief objective of the Diagnostic X-ray Department is to provide a complete diagnostic radiological service for the various institutes of the National Institutes of Health. Other objectives include the provision and operation of X-ray facilities for animal research; the operation of an isotope clinic providing diagnostic brain scans, thyroid uptake studies and thyroid scans; and the provision of advice and consultation on radiological problems.

Developments and Trends:

The rapidity of development and the trend toward electronic control of radiographic procedures continues to be somewhat amazing. Radiographic equipment is now appearing on the market utilizing remote controls no longer requiring the presence of a radiologist within the radiographic room to conduct fluoroscopic examinations such as barium enemas, G.I. Series, etc. Furthermore, electronic developments now permit the recording of X-ray images on magnetic tape for immediate play back and radiographic analysis. Such equipment will undoubtedly have a profound effect on the conduct of diagnostic radiologic procedures in the future.

Another new development has been that of elimination of wet film processing. There are now on the market at least six automatic film processing units which provide dry films for immediate interpretation within seven minutes. This has permitted exigency interpretation of dry films thus affording more accurate diagnoses and has at the same time eliminated the problem of handling wet films for immediate viewing. Such a unit, the Kodak X-Omat, has been installed within the Diagnostic X-ray Department.

The trend continues toward more extensive training requirements of radiological residents in order to qualify for board certification. To fulfill the requirements in this regard, the Diagnostic X-ray Department has inaugurated a four year residency training program. This program has received formal approval by the American Medical Association and by the American College of Radiology following inspection by members of these organizations.

Efforts are being continued to reduce radiation exposure to patients at the Clinical Center. A more sensitive scintillation counter has been purchased to conduct thyroid uptake studies, thus reducing radiation exposures. Higher kilovoltage techniques are used whenever possible which in turn permits reduction of radiation exposure to patients during radiographic studies.

As new products and radiographic substances are developed, they are continuously evaluated by the Diagnostic X-ray Department. This continuing study has led to the adoption of ditriokon as a contrast medium for the performance of angiocardiography. This new contrast agent is less toxic and better tolerated by patients.

Improvements and developments in motion picture recording of X-ray procedures have progressed to the stage where they are now practical and useful for research investigation. A cineradiographic unit has been obtained and its installation will be completed early in 1961. Many requests have already been received for the conduct of cooperative research studies utilizing this equipment.

Plans and Future Work:

Plans for the immediate future include the initiation of an X-ray technician training school within the department. The necessary approval and clearances have been obtained and it is anticipated that this program will be effective early in the calendar year 1961.

Radiographic equipment embodying television monitoring and a cineradiographic attachment is currently in the process of installation within the department. Future plans call for the utilization of this equipment for recording of physiological procedures. The ability to fluoroscope without darkroom adaptation utilizing this equipment widens the horizon of diagnostic services of the department.

The department plans to cooperate in every way in providing and operating the required radiographic equipment in the new surgical wing. There have been many changes in ideas and requirements on the part of the surgical teams as far as radiographic services are concerned in this area. Specialized procedures such as stereotaxic skull radiography will require specialized X-ray equipment and physical adaptation to the surgical rooms.

Providing suitable skilled personnel can be obtained, a more active role of the X-ray department is planned in the conduct of surgical radiological procedures. Every effort will be made to develop new techniques and to improve existing techniques to provide precise radiographic diagnoses.

A replacement program of old equipment is contemplated as it becomes obsolete and unsatisfactory in operation. New equipment will be carefully appraised as it is developed and only those changes which afford a real advantage over existing apparatus will be considered in the replacement program.

Future plans call for the continuation of consultation services of the department not only within the Clinical Center but to components of the Public Health Service and other governmental agencies. The teaching program within the Department will be somewhat expanded and joint teaching conferences with Walter Reed likewise will be supplemented in the near future.

Progress Achieved:

Important progress was made in the equipping of the department for the conduct of radiographic procedures. A cineradiographic unit with television monitoring was acquired during December of 1960 and is currently in the process of installation. Likewise a Kodak X-Omat film processing unit was installed and is now functioning quite satisfactorily. Finally, a two inch scintillation counter was purchased permitting more rapid handling of patients for thyroid uptake studies and at the same time affording a reduction of approximately fifty per cent in radiation exposure to the patient.

There was an increase in the number of X-ray procedures performed during 1960 from 33,606 in 1959 to 37,276 during 1960. This represents an increase in excess of 10 per cent in the total workload. There was a forty per cent increase in the number of surgical radiographic procedures performed with a new all time high of 553 such examinations.

Several new radiographic procedures, including gynecography and the conduct of thyroid scans, were added to the services provided by the department. The utilization of laminographic techniques for sectional radiography was appreciably expanded during 1960. A new contrast substance, Ditriokon, was adopted at the medium of choice for angiocardiography.

The technical staff was reorganized with the promotion of the Chief Technologist and the establishment of two assistant chief positions. One of the assistant chiefs will be responsible for direction of the X-ray technician training school while the other will have the primary responsibility for scheduling special radiographic procedures, assignment of work to the X-ray technicians and responsibility for smooth operation of the technical staff on a daily basis.

Official approval was obtained for the four year residency training program from the AMA and the American College of Radiology. Likewise, authorization and approval was obtained for the initiation of an X-ray technician training school.

Other achievements include the conduct of tours of the department for professional groups; the provision of consultation to various governmental agencies and other groups; presentation of technical papers at scientific meetings; and the showing of three exhibits at scientific meetings. The department was also repainted during calendar year 1960.

Major Problems Encountered:

As in preceding years, the chief problem of the department continued to be that of procurement and retention of skilled personnel. Lucrative salary offers outside the department continued to attract key technical personnel, primarily radiologists and technicians. Due to the routine and monotonous work involved in typing of X-ray reports, a turnover was experienced in dictating machine transcribers. Fewer applicants were available in 1960 as replacements and more than the usual amount of difficulty was experienced in obtaining this type of personnel.

Due to the construction of the new surgical wing on the Clinical Center, it was necessary to move the inactive X-ray files from the basement area. This posed several problems first as to procurement of suitable space for temporary storage of the films and secondly the expenditure of excessive time on the part of the film filing personnel in obtaining these old films as required when previous patients were readmitted to the Clinical Center. Likewise due to the increased workload, which seems to continue on a yearly basis, it has been necessary to move films from the active file sooner than desirable in order to obtain adequate space for films in daily use.

As was anticipated, there was a significant increase in the number of service calls required to maintain the radiographic equipment in satisfactory operating condition. The radiographic units are now approximately eight years old and the wear and tear on such equipment is beginning to become manifest.

A problem was encountered in the procurement of adequate nursing coverage of the X-ray department. The unpredictable demands for nursing service make this problem difficult to solve. After numerous joint conferences, it was decided that the best approach to this problem was that of the assignment of responsibility for nursing coverage to the surgical nursing service. The dependence of the X-ray department upon the surgical nursing staff has posed some continuing problems but this solution seems to be the most practical at present.

The acquisition of new equipment including the Kodak X-Omat unit and the new two inch scintillation counter presented operational difficulties early in their utilization. As additional experience was obtained, and following several service calls by the respective suppliers, the problems encountered with the operation of this new equipment gradually have been alleviated.

Honors, Awards and Publications:

An exhibit on "Filter Grids" presented at the American Society of X-ray Technicians meeting in Cincinnati received first prize. In addition, two other exhibits - one at the American Roentgen Ray Society meeting in Atlantic City and the other at the North American Radiological Society meeting in Chicago - were well received and obtained favorable mention.

The departmental chief presented the annual radiological lecture to the Maryland Radiological Society in Baltimore. He also presented a paper on the use of cineradiography and television monitoring in conjunction with X-ray equipment at the National TV Workshop held at the Naval Medical Center. Additional papers were presented by staff members at radiological meetings, conferences at Walter Reed, Johns Hopkins Hospital and at the Clinical Center.

The Chief Technologist of the department was appointed co-chairman of the Exhibit Committee of the forthcoming Third International Convention of X-ray Technicians to be held in Montreal, Canada.

There were seven papers published by staff members either as co-authors or as one of a series of authors during the calendar year 1960.

Calendar Year 1960

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

DENTAL DEPARTMENT

CC-36
Serial No.

As has been stated in previous annual reports, the objective of the Dental Department of the Clinical Center is to provide a diagnostic and treatment service for the various Institutes of the National Institutes of Health. The aim of the Dental Department is to perform a complete dental examination on every patient admitted to the Clinical Center. Recommendations for treatment are made to the attending physician and dental treatment is rendered for those patients for whom it is requested.

In previous annual reports, a number of examples of the type of cases which make the work of the Dental Department somewhat more difficult than that of similar facilities, have been cited. More recent examples may further clarify the important point; a young patient of the Mental Health Institute had not been able to receive dental treatment for many years. One of our dentists filled her teeth in the operating room under general anesthesia. In one 4 hour effort, twenty-one teeth were filled which included forty surfaces restored. Her teeth were also given a prophylaxis to improve oral hygiene.

A patient of the National Cancer Institute had a massive resection maxillae. A preoperative device was constructed to occlude the intra oral surgical defect which aided the nurses in feeding the patient and the patient in speaking. A new type of custom made sponge was devised to restore the continuity of this patient's oral cavity. This prosthetic innovation is being published in the literature.

A number of difficult cases were handled for the Human Genetics Section of the National Institute of Dental Research. A young lady with dentinogenesis imperfecta, all of whose teeth were stained and pitted, had to have every tooth capped with an esthetic and functional crown. Her morale benefitted considerably.

The Dental Department of the Clinical Center is continuing to cooperate with the Periodontal Investigations Branch of the National Institute

of Dental Research. Full mouth rehabilitation is being undertaken on a number of their cases in order to evaluate the part that restoration of occlusion and other factors play in the maintenance of teeth. This work is extensive and requires a tremendous amount of time and effort. The Training Program of the Periodontal Investigation Branch is particularly benefitted.

There are several other research projects which are being carried out by Clinical Center personnel and there are also many collaborative efforts with the staff of the National Institute of Dental Research. Examples are: (1) The biologic response of the human pulp to cavity preparation, (2) a cephalometric study of the position of the natural teeth and their supporting bone, as well as a similar study with the artificial substitutes, is being undertaken. (3) Studies of the temporomandibular joint syndrome. This was a new project instituted by Dr. Ronald Dubner, in collaboration with Dr. Paul Baer of the National Institute of Dental Research.

Several administrative changes were made in the Dental Department. A new type of filing system was installed. This was made necessary by the large number of records which have to be stored in the department. All dental progress notes are now hand written into the chart by each dentist where previously (all progress notes) were dictated. Dictation placed an impossible load on those personnel attempting to type on the records. We are now requiring all dental research outpatients as well as inpatients to have a comprehensive examination before they are admitted to a research project. This was thought necessary because of medical legal responsibilities in patient care.

The Dental Department is prepared to, and has rendered, every type of dental treatment found necessary for the patients examined. The following table documents statistics and is self-explanatory:

1959

Adm. to C. C.	Exams	Visits	Treatments
3400	1605	9152	10,838

1960

Adm. to C. C.	Exams	Visits	Treatments
3868	1682	10,308	11,086

There has been a comparable number of examinations performed; however, the number of visits have been increased. We are going to make

additional efforts to have a larger percentage of patients admitted to the National Institutes of Health referred to the Dental Department of the Clinical Center.

Calendar Year 1960

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

CLINICAL PATHOLOGY DEPARTMENT

CC-37
Serial No.

INTRODUCTION

Although this report includes activities and operations for the calendar year 1960, these statistics are for the fiscal year 1960 (July 1, 1959 to June 30, 1960). Statistical comparisons are between fiscal year 1959 and fiscal year 1960. The trends manifested, i.e., the rate of increase of workload and requirements for new tests have continued during the latter half of calendar year 1960.

MISSION

Experience in the Clinical Pathology Department for the past seven years emphasizes that opportunity for integrated research within the Department is essential. In addition to routine service, our mission involves adaptation of today's research for tomorrow's routine, a multi-disciplinary approach to clinical laboratory research and the training of pathologists. We have an exceptional opportunity to correlate physical, chemical and biological laboratory data for studying and understanding the complex reaction patterns of the whole patient.

Routine service operations necessarily were promoted during the earlier phase of clinical pathology growth in the Clinical Center. Very little research could be undertaken because of limited space and staff. We were unable to attract or hold those individuals most capable in clinical laboratory studies, who are able to recognize research potential of clinical problems and who are most likely to contribute to patient care. It is desirable for outstanding residents to remain for a few years after their training for further development and experience in this type of research.

If we are to retain such men in positions often financially inferior even to those in medical schools, they must have opportunity for research in our department. Adequate support for growth of our full research potential is essential, if we are not to fall behind medical schools and other centers and surrender our leadership in clinical pathology by default.

WORKLOAD

380,000 test procedures for research patient care were performed by a technical staff of 43 on 376 patients (daily averages). There were 97,900 patient workdays and the average workday load per technologist was 34.7. These figures represent an overall 10% increase in the laboratory work (33,680 tests) compared to 19% increase for the previous year. The

tendency to level-off is due to saturation of our capacity because of limited space and staff. We were unable to provide some 20 chemical test procedures which are frequently requested and should be available. Continuous contact with patient-care physicians by the residents and chiefs of our services indicate that there is very little, if any, duplication of tests or requests for unnecessary laboratory examinations. There may be an occasional "unneeded" test but this does not hazard a patient's welfare as does the omission of needed test. We are more concerned that we cannot provide all procedures needed for complete laboratory evaluation of patients in terms of modern knowledge and technical development. To accomplish this will require further space, more equipment, and some increase of personnel, in addition to systemization and automation of present operations.

Organizational Efficiency. The senior resident, supervisory technologist and Assistant Biochemist in Clinical Chemistry conducted an operational study of work flow, staff utilization and technical requirements of test procedures. To correct inefficiencies, we "unitized" groups of technically similar tests, organized the technologists into teams responsible for these blocks of tests and changed personnel rotation so that transfers of technologists from one team to another are only one at a time. Space reorganization improved physical relations of requipment and functions in the flow of work in the unitized system. These changes promoted efficiency, permitted a little more time for several new tests and encouraged all personnel. Similar studies have been initiated in Hematology and Microbiology Services.

These innovations plus automation of several additional procedures increased the average productivity of our technical staff, and reduced the more tedious and fatiguing operations which are being taken over more and more by machines. However, several prolonged periods of peak workload were weathered only by the untiring efforts, loyalty and long overtime work of our excellent staff. It is encouraging that these periods were not as frequent or long and the pressure not quite as severe as in previous years. Thus, the average optimum capacity now is estimated to be 30 tests per technologist per workday.

Quality Control has been continued, but we have been unable to afford the personnel and time to extend control to newer tests and to improve quality control as much as desirable. Nevertheless, our original control program has provided for discovering most errors before they become a part of the patient's record, and providing confidence in our work.

PERSONNEL

The new standards of classification for technologists (professional) and technicians issued by the Civil Service Commission have been very successfully applied to the benefit of our staff. All but 6 of our technical staff who were formerly necessarily classified as chemists and bacteriologists are now medical technologists. 20% of our technician staff qualified professionally for the new rate and 7 qualified for promotion to GS-9 in supervisory positions during the past 18 months.

The following promotions were earned and accomplished during 1960:

1 Bacteriologist	From	GS-5	To	GS-7
1 Bacteriologist		GS-7		GS-9
1 Bacteriologist		GS-9		GS-11
1 Bacteriologist		GS-11		GS-12
1 Medical Technician		GS-5		GS-6
1 Medical Technician		GS-6		GS-7
2 Medical Technicians		GS-6		GS-7 (Technologist)
1 Medical Technician		GS-5		GS-7 (Technologist)
2 Medical Technologists		GS-5		GS-7
1 Medical Technologist		GS-7		GS-9
1 Chemist		GS-5		GS-7
1 Chemist		GS-9		GS-11

Attrition is minimal, and chiefly due to maternity or moves with family to another city.

1 Resignation to accept better salary
2 Resignations for maternity reasons
4 Resignations to move to another city
1 Resignation to start own business

Staff. Our Visiting Scientist in Clinical Chemistry, Dr. Gunnar Jungner, Professor Clinical Chemistry, University of Gothenberg, completed his tour with us and returned to Sweden in September 1960. He contributed many ideas, new procedures, suggestions for automation; his deep knowledge of chemistry, wide experience and understanding nature earned him the affection of our entire staff, as well as providing valuable scientific leadership.

Dr. Gareth P. Gladstone, Reader in Bacteriology, Sir William Dunn School of Pathology, University of Oxford, England, joined the Department as Visiting Scientist in Microbiology on July 1, 1960.

Dr. Iain MacIntyre, Lecturer in Chemical Pathology, Hammersmith Hospital, Postgraduate Medical School, University of London, arrived December 23, 1960, for a nine month tour as Visiting Scientist in Clinical Chemistry. He brings a broad experience and excellent training from one of the outstanding departments in the world.

Dr. F. W. Sunderman, Jr., became Acting Chief of Clinical Chemistry on July 1, 1960 and resigned effective December 31, 1960 to return to Jefferson Medical School and devote his full time to research under his father. His proposals for reevaluation of a technical method each month and increased resident instruction were sound; however, Dr. Sunderman avoided the responsibility of solving many daily problems, even those arising from the adoption of some of his methods. He pursued his own research to the exclusion of his service responsibilities, isolated himself from his staff and took little interest in the problems of the patients. As a result, morale in the Clinical Chemistry Laboratory deteriorated until his departure.

Dr. Philip Geisler was appointed Assistant to the Chief of Clinical Pathology effective July 1, 1960.

Dr. Frederick A. Fox resigned as Acting Chief, Clinical Chemistry, June 30, 1960, to accept appointment as Director of Clinical Pathology of the Miller Hospital at St. Paul, Minnesota.

Dr. H. H. Marsh completed his third year in clinical pathology and transferred for his final year for pathologic anatomy to the Laboratory of Pathology and Histochemistry, NIAMD.

Dr. Robert Bell completed his second year of Clinical Pathology and moved to group practice in Indiana on July 1. Dr. Andrew Tegeris completed two years in Clinical Pathology and transferred to Pathologic Anatomy Department, Clinical Center.

Drs. Geoffrey Brittin, Thomas Liddy, Coleman Seskind and Harold Varon joined the resident staff on July 1, 1960; Drs. Robert Fouty and Rowan Boylan remained as senior residents in Clinical Pathology.

Dr. Robert Fouty, as Senior Resident in Clinical Chemistry during the last six months of calendar year 1960, has been outstanding in his leadership and ability to work with the technologists and the patient-care physicians. He has contributed many ideas for the systemization and increased efficiency of the clinical chemistry operations, and has been very effective with Mr. Nishi in assisting and encouraging technical staff.

OPERATIONS

Automation. The successful automation of several chemical analyses by the autoanalyzer, amperometer and recording colorimeters, and of leukocyte and erythrocyte enumeration with the high speed electronic counter, led to a more comprehensive study of the feasibility and benefits of extensive automation and systemization so that our capacity for work volume and speed could be significantly increased. The marked acceleration of scientific technology and medical research is producing a parallel growth in applicable laboratory routine which will demand tremendous increases in laboratory capacity for work and speed. Normal 15% increase per year (experience of 5 years) doubled the load in 4 years; but the accelerating pace of research and application will again double this load within 5 years. Therefore, we must develop mechanization and automation, decrease amounts of specimen required for each analysis (especially blood) and substitute rapid methods of analysis for many of the traditional slower technics and thus expand our productive capacity ten fold by 1970.

The large amounts of laboratory data related to each patient will require correlation into patterns by computer data processing methods. A pilot project already under development will provide a four channel automatic system which will identify the specimen, distribute aliquots, complete analytical reactions, transfer the color reaction mixtures sequentially through a high speed digital direct-read-out colorimeter and print out the results from a computer. The most important component

of this chain is the digital read-out sensing device (colorimeter). There are none available on the market. By contract, Bolt, Beranek & Newman, are providing the engineering and design aspects in designing and producing a working model of this instrument. The first "bread board" fabrication was delivered for evaluation a few weeks ago, but there has been delay in negotiating renewal of the contract, so work on this important phase of our program is temporarily suspended. The attached chart depicts the block and flow diagram of the components and function of this four channel system.

Clinical Chemistry

Summary. Clinical Chemistry carries half of the total test load of clinical pathology, i.e., 188,444 or 49% for fiscal year 1960 with 40% of the technical staff. Automation and tests being performed in batches make this possible. However, the technical staff in chemistry is most severely loaded and space limitation has become acute.

Improvements in organization and work-flow. Dr. Frederick Fox initiated reorganization of staff and work operations into functional units and this was further developed by Dr. Robert Fouty, Mr. Nishi, Miss Pastewka and Miss Bunting. Improvements are: (1) decrease of time lost in unnecessary movements of the technical staff about the department; (2) consolidation of instruments and supplies into functional units; and, (3) assignment of technical staff in teams. All urinalysis is done in one room, eliminating unnecessary transportation of specimens between laboratories; all electrolyte determinations are located together and performed by an "electrolyte team". Thus, the time for serum sodium, potassium, chloride and carbon dixoide content has been reduced to approximately 45 minutes, a marked advantage to prompt and emergency patient-care. Efficiency was improved still more by similar changes in liver function tests, autoanalyzer operations, ultra micro analyses, etc.

New procedures initiated: Blood volume measurement by Evans Blue, (as accurate a method as the radio-chromium, but without the radiation hazard); determination of 3-methoxy 4-hydroxy mandelic acid in urine (instead of catecholamines), for the diagnosis of pheochromocytoma; and qualitative determination of urinary myoglobin using paper electrophoresis. These are three of more than 20 new tests which should be available for patient care.

Blood pH determinations have been greatly improved in sensitivity, speed of performance (bedside procedure) and convenient by the new Metrohm instrument. Dr. Robert Fouty initiated this project with enthusiasm and did an outstanding job of calibrating the instrument and evaluating its clinical utility. From the blood pH and carbon dioxide content, the carbon dioxide tension (pCO^2) is calculated to provide clincians with more meaningful information regarding the acid-base status of their patients.

Developmental Projects. Because of the growing number of chemical determinations required on blood we are evaluating the ultra micro methods (SANZ, Geneva), requiring less than 0.5 ml. of blood per test. The

calcium method is reliable and rapid. Three other methods have proved useful. Completion of this study will make available ultra micro determinations of blood glucose, urea nitrogen, total protein, cholesterol, calcium, creatinine, and uric acid, particularly important for care of leukemic children.

Macro serum calcium measurements are tedious. Mr. Nishi has developed a high speed system for flow of specimens into a chart recording flame photometer at a rate of 1 every 6-10 seconds. The method is apparently accurate. Dr. Fouty is working on a simplified enzymatic determination of serum pyruvate, which will contribute to the differential diagnosis of peripheral neuritis. Dr. Ness is exploring the possibility of a simple rapid method for total iron binding capacity, using Nitroso R.

Special Problems. Shortage of space is acute in clinical chemistry. Space is urgently needed for many additional analytical procedures required for up-to-date patient care, developmental work in automation, and laboratories for residency training. Dr. Geisler, Assistant to the Chief of this Department, has a desk in one corner of a laboratory.

Night utilization of laboratories. Some of the more important tests needed are lactate acid dehydrogenase, serum glutamic pyruvic transaminase, vitamin A tolerance, serum carotene levels, 2 dimensional paper chromatography of urinary amino acids, etc. Therefore, it has become necessary to start utilizing laboratory space at night. We have assigned one technologist full time from 4 p.m. to 12:30 a.m. and will employ a second night technologist in January 1961. Night operation permits more efficient utilization of space and equipment will help increase our work capacity, and will relieve day staff of several procedures, results of which are not immediately necessary. Some developmental work and special tests also can be done at night.

Equipment Problems. The heavy demands upon our laboratory equipment has necessitated the addition of a full-time technician (instrument engineer) responsible for maintenance and repair. Mr. Eve, GS-7 physicist, has instituted preventive maintenance of electrical equipment and thus decreased the breakdown time which is one of the factors interfering with efficient production. He has also developed many needed devices to improve laboratory operations and is contributing to our automation program. He is well qualified for promotion to GS-9.

At the present time there are a number of instruments needed for efficient and more comprehensive function of our clinical chemistry service. These are listed as needed new equipment and equipment to replace worn out or obsolete apparatus.

1 Beckman B D Spectrophotometer with Recorder	$ 3,000
1 Autoanalyzer	5,000
2 Recorders	2,000
1 Ultramicro System	1,100
1 Oscilloscope	1,000

2 Centrifuges	$ 1,000
1 Balance	700
1 Calculating Machine	500

Visiting Scientist in Clinical Chemistry

Dr. Gunnar Jungner contributed important improvements in technics, new test procedures, and almost daily ideas to improve organization, efficiency of work and modification of instruments. He inspired the entire staff with his keen interest, profound knowledge and enthusiasm. He participated actively and willing in advising adminstrative policies from his personal experience as a department head and helped train technologists and residents.

Dr. Jungner developed an accurate micro method for analyzing CO_2 on the autoanalyzer, adapted the transaminase procedure to automation, and development of other enzyme analyses (amylase, pyruvic transaminase and lactice dehydr genase) for automatic instruments. He demonstrated the usefulness of such automatic and highly sensitive micro methods for use in measuring the metabolic and other differences of microorganisms.

He is preparing several manuscripts for publication in the near future.

We are making every effort to persude him to return and join our permanent staff as Chief of Clinical Pathology Research.

Hematology Service

Summary. The work volume in Hematology for FY 1960 was 30% of the total for Clinical Pathology with 36% of the technical staff.

During the months June to November 1960, inclusive, 65,600 tests were performed in the Hematology Service, an increase of 4,700 tests, or nearly 8% over the corresponding period of 1959. Furthermore, we had to absorb a temporary reduction in personnel (3) due to maternity leave. This seriously interfered with the development of new methods and full utilization of the experimental Coulter plotter equipment now available. Progress in all developmental projects therefore has been distressingly slow. Recruitment of additional personnel has become increasingly difficult due to the limited number of well-qualified technologists available. It is gratifying that we have had no resignations from Hematology since the new standards for technologists (professional) effected comparable status with other laboratories in this area.

Under these circumstances, our greatest need is for a developmental research unit within the Hematology Service with at least one additional staff member and three technologists consistently to explore and develop new technics and automation and to avoid stagnation.

There is an acute need for an electron microscope. It has become obvious from light microscopic and phase microscopic studies of the 50 or more new cases of leukemia each year, that classification into lymphocytic

and myelogenous types of acute leukemia is of considerable clinical importance as well as academic interest. Response to corticoids, serum protein changes and other parameters showed distinct differences between the two types of leukemias. Classification of type was made in Hematology on repeated morphologic studies without knowledge of the other parameters mentioned. Chemical and morphological evidence strongly suggests exploration by the electron microscopy will reveal further sub-classification of these leukemias which will permit further and more meaningful clinical correlation, especially with therapy. Such morphologic changes now reported simply as "vacuolization", need to be explored for chemical composition with histochemical and centrifugation technics.

New Procedures and Development

The accuracy and speed of hemoglobin determinations have been much improved by introduction of flow-through cuvettes. This dispensed with the need for large numbers of special expensive colorimeter tubes economizing on their purchase, washing and breakage. The new procedure eliminates the time consuming calibration of these special tubes. Further modification of our Klett colorimeter by adding an electronic null increased the speed of reading galvonometer individual tests and eliminated fatigue. This contribution was the work of Dr. Marsh.

An increase in services of particular interest during the past year has resulted from the need for additional platelet counts to assess the status of patients receiving 2 and occasionally 3 platelet transfusions per week. These frequent transfusions are made possible by use of the same donor, with return of red cells to the donor after separation of the platelet rich plasma. Because only fresh platelets will circulate, and there is often a temporary sequestration of transfused platelets with their subsequent reappearance in the circulation, it was necessary to do platelet counts for several hours following each transfusion. The procurement and processing of platelet rich plasma prevents transfusion before early afternoon; therefore, considerable overtime work was required to follow these patients. Although this collaborative project with the leukemic service has resulted in much new information, and might be considered "research," frequent platelet counts are needed for evaluation of the patient's status and when this type of donor plasmapharesis has become routine, frequent platelet counts will continue to be necessary for proper care of the patient. The anticipated immunization against donor platelets did not occur and the multiple platelet transfusions have successfully maintained platelet levels above the minimum at which clinical bleeding develops.

Investigations of red cell sizing and platelet counting with the Coulter Counter have been among the major projects. Both projects have been greatly aided by the purchase of a graph-out device (particle size plotter) for the Model B Coulter Counter. This equipment traces a graph of cell size distribution in a few minutes for each sample. A suitable diluent which does not change red cell volume was developed to preserve cell volume as well as plasma which is presumed to maintain red cell size

as it exists in vivo. Preliminary results of clinical applications were presented at a meeting of the Clinical Pathologists in September 1960.

Recovery from iron deficiency, characterized by small (microcytic) cells, could be followed by observing the shift of size peaks from a single population of microcytes, to a mixed population of normal cells and surviving microcytes (two peaks) and finally to a totally monocytic normal population. Mixing experiments indicate that in case of relapse, as few as 10% of microcytes can be picked up in the graphs of cell size distribution, a number of which may be insufficient to lower the average values for the mean cell volume (MCV). sufficiently to be diagnostic. Continued graphing of red cell distribution, therefore, may permit earlier detection of microcytic anemia than any other available technic.

Attempts to automate platelet counts with the Coulter Counter have not yet yielded a practical technic for routine use, but some progress has been made in defining the conditions necessary for accurate platelet counting. A comparative study of leukemia and infectious mononucleosis was initiated to determine any differences which might exist in the DNA synthesis of the two types of cells to assist in differential diagnosis. In vitro labeling the radioactive thymidine was utilized and a high proportion of cells of infectious mononucleosis were found capable of DNA synthesis.

Personnel and Space

A majority of test procedures in Hematology require the personal attention of an adequately trained, knowledgeable and skillful technologist and meticulous, often dexterous manipulation. Tests are not as readily automated or mechanized as in chemistry. Therefore, the technical work capacity of the staff expressed in terms of tests/technologist/day is not identical to either the average for the entire department or Clinical Chemistry.

During FY 1960 the daily load averaged 27.8 tests per technologist. Our inability to introduce all needed new tests and progress satisfactorily with development and evaluation indicates that this load is too high. We estimate that the optimum capacity for efficiency and to support proper progress at the present state of automation is 400 tests per day. At the moment, we have one technologist working part time on both red cell sizing and developmental work on automatic platelet counting and progress is severely limited by this lack of rapid utilization of the automatic equipment now on hand. Even simpler aids to clinicians must be left unexplored because we lack the personnel to evaluate new methods before we can introduce them for routine use. For instance, actual counting of siderocytes among normoblasts probably would be a very accurate measure of iron stores and much superior to the staining of hemosiderin within reticulum cells as done now. Similarly, we cannot perfect micromethods for L. E. preparations which would be applicable to serum as well as whole blood.

Space requirements include 4 modules for Hematology: 1 for the Assist- and Hematologist, 1 for electron microscopy, 1 for residents (2), and 1 for

expanded automation and development.

Equipment

Centrifuge	$ 600.00
Micro-Centrifuge (Drummond)	165.00
Microscopes, A.O., Spencer (2)	1,388.00
Electric Pumps (2)	50.00
Water Bath	92.00
Counters - Clay-Adams (2)	114.00
Brewer Automatic Pipetting Machine (2)	990.00
Model C Coulter Counter	4500.00
Thermo-Fax Copying Machine	489.00

Microbiology Service

Ten technologists performed 62,950 microbiological procedures during FY 1960, 17% of the total volume in Clinical Pathology. The professional caliber of our technologists is well illustrated by an example - a technologist experimented with various dilutions of organisms at varying rates of growth to determine the optimum combination for sensitivity tests. An unavoidable delay after the infectious agent is isolated from culture is determination of its antibiotic sensitivity pattern. Another technologist collaborated with Dr. Kimler, Media Section, in developing a medium for rapid growth and visible recognition of the organism. At our request Baltimore Biologics Laboratory has prepared an experimental batch of high concentration penicillin discs (25 & 50 units each; 10-fold increase over highest available) for study of penicillin sensitivity related to therapeutic response.

The standard method of laboratory diagnosis of M. tuberculosis is usually by growth of the organism on special media and its pathogenicity for guinea pigs. With the onset of INH therapy, however, more atypical forms of the organism have been observed which may not be pathogenic for laboratory animals. Various chemical tests have been reported in the literature which indicate differences between atypical (but probably pathogenic) organisms and the true M. tuberculosis v human. We have tested all these procedures and have incorporated the most useful and reliable into our routine armamentarium. Thus, more precise information as to the true nature of the organism is now available to the clinician.

Under the excellent guidance of Dr. Gareth Gladstone (Visiting Scientist), we are evaluating and adapting fluorescent antibody methods to diagnostic bacteriology and serology.

Phage Typing of Staphylococci

During the past year Mr. Zierdt has further streamlined this test by using only one page concentration, instead of two. This is 100-fold the concentration of the "Routine Test Dose" (RTD) customarily employed. It eliminated false reactions and types a higher proportion of organisms.

Automatic phage applicators duplicating the one developed here are now in use in this country and abroad. Phage typing demonstrations were presented before the Maryland branch of the American Society of Clinical Pathologists, before the Medical Technology Socieyt (ASCP), and to numerous visitors to NIH. Mr. Zierdt has furnished advice, and in some cases materials and training to those setting up phage typing in other laboratories. These include Fort Meade, National Naval Medical Center, Food and Drug Administration, Georgetown University, State University of New York, University of Maine, George Washington University, and others.

Dissociation in Pseudomonas Aeruginosa

The identification of pseudomonas is indefinite and annoying. Under conditions best demonstrating natural mutations and colony phenotypes, 121 resultant dissociants were obtained from 14 initial colony types by Mr. Zierdt in his extensive work with this organism. These were subjected to 25 biochemical and morphological examinations designed to reveal differences beyond colony morphology. Tentatively, the results demonstrate that phenotypic colony dissociants of a single parent type may be different organisms biochemically.

The Triple Sugar Iron Agar, Urea Agar, Simmon's Citrate, synthetic dextrose, and 10% lactose media used in the routine laboratory for Pseudomonas detection often leave the bacteriologist in doubt. The application of these results promise to facilitate the identification of Gram negative bacilli in the diagnostic laboratory.

Culture of Pneumocystis Carinii

The nature of the etiologic agent of pneumocystis pneumonitis (interstitial plasma cell pneumonitis), a disease of increasing importance, is unknown. His experience and knowledge of tissue culture technic qualified Mr. Zierdt to collaborate with Dr. H. H. Marsh and Dr. Gert Laqueur, NIAMD, in attempts to culture Pneumocystis carinii. A yeast-like organism has been maintained in culture from infected rat lungs. Germ free animals will be utilized for further trials.

Visiting Scientist in Microbiology

Dr. G. P. Gladstone joined the Microbiology Service on July 1, 1960. With technical assistance provided by the Department, he has developed a rapid method for the assay of anti-staphylococcal leucocidin in human serum which can be used for routine diagnosis. He is applying the test to survey the serum antibody titers of Clinical Center patients with staphylococcal disease, and of carriers and normal individuals. There is evidence that the test may have diagnostic value in cases of deep seated infection in which no organism has been isolated. He is collaborating with Dr. Stuart Mudd of the Veterans Hospital and University of Pennsylvania Medical School, Philadelphia, in an assessment of the value of the vaccines in Staphylococcal infection.

In collaboration with Mr. Zierdt, Dr. Gladstone also is investigating the action of a cytophathogenic toxin produced by Staphylococci on Hela tissue culture cells and its neutralization by antiserum and the serum of patients with staphylococcal disease.

Contributions to Patient-Care

The Microbiology Service often works in conjunction with other institutes in special research studies especially pertinent to patient-care. To reduce morbidity of infections following extensive surgery, we are collaborating with the Cancer Institute in a thorough study of the sources of postoperative infections.

We are collaborating with the Laboratory of Clinical Investigation, NIAID, in a bacteriological study of normal human volunteers.

The Microbiology Service prepares a weekly Infections Report of patients and employees for the Clinical Center Infections Committee. The clinical pathology resident in Microbiology reviews daily all laboratory reports and records those patients with pathogenic organisms. Each case is reviewed by him with the patient's physician, if necessary, and the status of infection is recorded on the report. In this way the Infections Committee maintains close surveillance of the occurrence of infections, discovers the spread and source of any hospital acquired infections, and can control them before epidemics occur.

In-Service Training of Personnel

Residency training on Microbiology Service is similar to that in the other Services including rotation through each of the laboratories to learn every phase of the work, discussions with the Chief of Service one hour each week, and conduct a formal class in theoretical aspects of bacteriology and immunology. They are responsible for preparing case reviews for semi-weekly Laboratory Rounds in the department, and for preparing a formal paper each week for presentation to the Microbiology staff such as a case presentation, a review of literature or a seminar participated in by the technologists. These weekly "classes" in which the Chief of Service and Visiting Scientist also participate supplement the general program of personal daily instruction of the technical staff in advanced methods and theory.

For a period of three months each, two technologists were detailed to the Laboratory of Clinical Investigation, NIAID, to learn advanced methods and identification in parasitology. Our Chief Supervisory technologist was sent to the Mycology Service at the Walter Reed Army Institute for Research for one month for special training in complement fixation tests for fungal diseases.

Equipment

3 Microscopes	$ 1,200.00
1 Vacuum Pump	215.00

1 Incubator, bench	$ 500.00
1 Refrigerator	300.00

Summary of Space Requirements in Clinical Pathology Services

	Modules
Office of Chief	
Assistant to Chief	1
Clinical Chemistry	
Chief Supervisory Technologist	1/2
Evaluation of new procedures	1
Automation development	1
Residents	1/2
Additional patient care analyses	1
Hematology	
Assistant Hematologist	1
Electron microscope	1
Residents	1
Automation and new test development	1
Microbiology	
Visiting Scientist	1
Automation development	1
Chief of Service	1
	12

These modules are needed to relieve the crowded conditions and provide for efficient development of automation. For instance, in Microbiology the Acting Chief, Secretary, Resident and Chief Supervisory Technologist all occupy the same module. In Hematology, the new test development, the Assistant Hematologist and Secretary and Clerk all occupy the same module. There is no other place to put them!

Awards

None

Publications

The Hematologic Toxicity of Pyrimethamine (Daraprim) in Man; Kaufman, Herbert E., M.D. and Geisler, Philip H., M.D. A.M.A.Archives of Ophthalmology, Vol. 64, July 1960.

A Multiple-Syringe Bacteriophage Applicator; Zierdt, C.H., M.S., Fox, Frederick A., M.D., and Norris, George F. Am.J.Clin.Path. 33(3):233-237, 1960.

Fever in Malignant Neoplastic Disease: A Controlled Study of Tetracycline Therapy; Boggs, Dave R., M.D., Frei, Emil, M.D., and Zierdt, Charles H., M.S. Annals of Internal Medicine, 53(4):754-764, 1960.

Ultraviolet Studies of Tetrazolium Reduction in Living Cells; Williams, G.Z., M.D. and Peacock, A.C., Ph.D. Experimental Cell Research, Suppl. 7, 131, Nov. (1959).

Rapid Electronic Measurement of Cell Volume and Distribution; Peacock, A.C., Ph.D., Williams, G.Z., M.D., and Mengoli, H.F., Ph.D. J.Nat.Cancer Inst. 25:63, July (1960).

Paper Electrophoresis; Fouty, R.A., M.D. Maryland State Journal, Dec. 1960.

Survey of Staphylococci Types in Hospitals in the District of Columbia, Maryland and Virginia; Marsh, H.H., M.D., and Hicklin, M., M.D. Med.Ann. of D. C. 29:214-218, 1960.

Enzymes in Liver Disease; Fox, Frederick A., M.D. Med.Ann. of D.C. 1960.

CLINICAL PATHOLOGY DEPARTMENT

Fiscal Year 1960

	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	Avge	Total
Av Pt Census	395.5	383.5	375.0	372.7	390.0	344	387	410.0	378.0	382.8	383.4	403.7	375.5	4,506
Pt Wkdys	9,096	8,153	7,875	8,199	7,410	7,568	7,740	8,200	8,694	8,039	8,051	8,881	8,159	97,906
Test Proced	30,178	27,949	29,417	28,936	28,345	27,843	30,913	35,511	35,762	34,483	34,730	35,919	31,665	379,986
Test/Pt/ Wkdy	3.5	3.3	3.7	3.5	3.8	3.6	3.9	4.3	4.2	4.2	4.3	3.9	3.9	46.2
Tests/ Pt	74.2	71.0	76.6	76.1	71.5	79.4	78.7	86.6	94.6	94.6	88.7	86.8	81.6	978.8
Total Staff	50	50	48	51	51	51	52	52	52	49	47	47	50	600
Test.Tech /Day	32.9	31.2	33.7	29.5	32.6	30.5	33.5	38.5	34.0	39.0	40.7	40.4	34.7	416.5
Total Equiv. Tech.	43.6	42.7	41.6	41.7	45.7	41.5	46.2	46.1	46.0	42.1	40.6	40.4	43.4	521.2

1959-60 Fiscal Year

Average Tests/Patient

	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	Avge	Total
NCI	115.0	111.6	107.1	106.8	103.5	110.7	116.1	126.4	133.7	137.8	134.6	132.9	119.7	1,436
NIAID	128.3	111.0	143.8	120.8	123.2	146.1	158.5	153.5	157.2	201.2	176.9	128.5	145.8	1,749
NHI	86.8	72.0	79.9	89.1	83.6	83.1	74.3	90.2	87.7	82.7	84.9	77.7	82.7	992
NIAMD	63.9	63.9	73.9	78.2	65.7	72.5	78.8	81.6	99.8	71.8	81.4	80.3	76.0	912
NINDB	33.9	35.8	36.1	30.0	28.0	43.2	51.3	49.2	59.4	52.6	43.4	54.2	43.1	517
NIMH	3.9	6.8	17.2	10.5	6.7	9.8	13.3	12.2	21.4	10.7	10.4	16.7	11.7	140
NIDR	60.0	68.6	116.5	90.0	92.2	148.9	96.8	86.4	156.0	236.6	316.6	264.6	114.4	1,733

CLINICAL PATHOLOGY DEPARTMENT

Chief, Dr. George Z. Williams
Asst to Chief, Dr. Philip H. Geisler

Secretary - Mrs. Vivian Arnold
Asst Secy - Mrs. Elizabeth Smith

3 Departmental Laboratory Aides

Clinical Chemistry Service	Hematology Service	Microbiology Service	Clinical Pathology Developmental Research Laboratory (NCI)
Acting Chief,	Chief,	Acting Chief,	Chief,
Dr. George Z. Williams	Dr. George Brecher	Dr. Edna Schnaper	Dr. Andrew C. Peacock

CLINICAL CHEMISTRY SERVICE

Acting Chief, Dr. G. Z. Williams

Secretary - Mrs. Ada Jones
Clerk-Typist - Mrs. Panola Brach

Chemist for Development

Dr. Arthur T. Ness

Laboratory of Electrophoresis & Chromatography

Dr. Henry C. Dickerson

Visiting Scientist

Dr. Iain MacIntyre

Senior Residents

Dr. Robert Fouty
Dr. Rowan Boylan

Jr. Resident

Assistant Biochemist

Mr. Hiroshi Nishi

1 Technologist

Chief Supervising Technologist

Miss Jullia Pastewka

Supervising Technologists

Miss Sylvia Bunting
Miss Miriam Bowman

17 Technologists
1 Part-time Technologist

Ultra-Micro Chemistry

Mr. L. T. Byrd

HEMATOLOGY SERVICE

Chief, Dr. George Brecher

Secretary - Mrs. Helen Fremming
Clerk-Typist - Mrs. Jean DeMarco

Fellow - Dr. J. Russell Little

Jr. Resident

Chief Supervising Technologist

Miss Minna Feld

Supervising Technologist

Miss Mary E. Cummings

16 Technologists
2 Laboratory Aides

MICROBIOLOGY SERVICE

Acting Chief, Dr. Edna Schnaper

Secretary - Mrs. Martyle Auman

Jr. Resident

Chief Supervising Technologist

Miss Anne Jennings

Supervising Technologist

9 Technologists

1 Laboratory Aide

Laboratory of Phage Typing

Mr. Charles H. Zierdt

Visiting Scientist

Dr. Gareth Gladstone

Bacteriologist,

Mr. H. D. Hochstein

Developmental Bacteriology

Chief Technologist

Mrs. Delores Kenton

CLINICAL PATHOLOGY DEVELOPMENTAL RESEARCH
LABORATORY (NCI)

Chief, Dr. Andrew C. Peacock

Chemist

Mr. Roswell Reed

Bacteriologist

Mr. Howard Bladen

3 Chemists

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

REHABILITATION DEPARTMENT

CC-38
Serial No.

The Rehabilitation Department during Calendar Year 1960, as in previous years, continued to carry out its basic function of patient treatment, using recognized, effective measures of physical, occupational, and speech therapy. Again, a large number and wide variety of tests and measurements were carried out for Institute physicians, primarily for evaluation of functional ability and progress, but often in connection with diagnosis and determination of effectiveness of drug and other therapy.

The numerous specific services provided by the Department are listed in detail in the Summary Annual Report for Calendar Year 1959 (pages 1-3) and are not repeated here in the interest of economy of space.

SPECIAL PROGRAMS:

A number of special programs, begun in previous years, in which the Department actively participated in Institute projects, were carried on during calendar year 1960:

1. Continued evaluation of the use of plaster casts and splints in the treatment of patients having rheumatoid arthritis.

2. Continued weekly measurements of joint range of motion and determination of joint range percentage deficits for rheumatoid arthritis patients on drug studies.

3. Continued pre- and postoperative evaluation and treatment of heart surgery patients. These patient referrals are primarily concerned with preoperative orientation and postoperative treatment to prevent loss of range of motion and help the patient achieve normal functional capacity. A paper covering the treatment of 682 patients from July, 1954 through June, 1960 is being prepared for publication.

4. The handbook for arthritis patients on which Rehabilitation Department personnel had been working was published in calendar year 1960. Entitled "Strike Back at Arthritis" (Public Health Service Publication #747), it was presented at the Annual Meeting of the American Medical Association in Miami Beach, Florida, in June, 1960.

Special programs which were begun in calendar year 1960 include:

1. Pre- and postoperative evaluation of patients having neurologic disorders considered appropriate for stereotaxic operative procedures. Collaboration of Rehabilitation Department personnel and the Neurosurgical Staff has resulted in reasonably objective tests of functional activities. These tests, together with determination of muscle strength and joint range of motion measurements conducted pre- and postoperatively on each referred patient, are of aid in the evaluation of the postoperative status and surgical results. This program is recently established, and its expansion is anticipated. In calendar year 1960, fifteen patients were evaluated in the Rehabilitation Department.

2. Treatment of patients having cystic fibrosis using breathing exercises and postural drainage technics.

3. Evaluation of specific therapeutic measures in patients having psoriatic arthritis, including measurements of range of motion of joints of the fingers of both hands.

STAFF TRAINING:

In June, 1960 Mr. Ronnie Townsend, Assistant Therapist, attended a one-week course in Physical Therapy in the Treatment of Cystic Fibrosis at Boston Children's Hospital Center.

Also in June, 1960 Miss Heidi Nadel, Speech Therapist, attended a one-week postgraduate course in Speech and Voice Disorders at the University of Miami School of Medicine in Miami, Florida.

In July, 1960 a one-week course in Proprioceptive Neuromuscular Technics and Patterns was conducted at NIH by Miss Margaret Knott, Chief Physical Therapist at the California Rehabilitation Center, Vallejo, California. Sponsored by the

Rehabilitation Department, it was attended by the entire Department staff, as well as by 14 physical therapists representing major military and civilian hospitals of the Metropolitan Washington area and Baltimore.

Throughout calendar year 1960, the Department provided orientation and training programs for its new staff members, and also participated in in-service training courses, orientation lectures, and Rehabilitation Department tours for a wide variety of Clinical Center personnel, including Practical Nurses, Gray Ladies, Head Nurses, Social Service staff, Nurse Officers, Clinical Pathology staff, and others.

The Rehabilitation Department was selected as one of a number of service and research areas whose activities were kinescoped for subsequent closed circuit television for newly arrived NIH physicians. Rehabilitation goals were described, equipment and procedures were shown and patients presented.

The Department subsequently participated in a similar manner in the production of a documentary film "American Medicine" by the well-known West German telecaster, Peter von Zahn, for viewing by European audiences.

PUBLICATIONS, EXHIBITS, AWARDS:

1. "Strike Back at Arthritis" (PHS publication #747). Dr. David M. Fried, Chief of the Rehabilitation Department, was a member of the Editorial Committee, and Miss Vida Jo Niebuhr, Chief of the Physical Therapy Service, organized the exercise sequences and other physical therapy procedures. The photographs were made in the Rehabilitation Department under the supervision of Department personnel.

2. Case histories were published in the Physical Therapy Review, official journal of the American Physical Therapy Association:

   July 1960: Use of Plaster Casts for Correction of Severe Equinus Deformities, by Vida Jo Niebuhr.

   October 1960: Hemiplegia Following Open Heart Surgery, by Vida Jo Niebuhr and Jane Blue Holleman.

3. The NIH exhibit, Plaster Casts and Splints in the Treatment of Patients Having Rheumatoid Arthritis, was presented at the American Medical Association Annual Meeting in Miami Beach, Florida, June 13-17, 1960 by Dr. David M. Fried, Chief of the Rehabilitation

Department, Mr. Mario Salvanelli, Assistant Chief of the Physical Therapy Service, and Miss Vida Jo Niebuhr, Chief of the Physical Therapy Service. Demonstrations of cast and splint application were given twice daily. Literature was made available describing the purpose of certain forms of splints and giving detailed instructions for their application.

4. The Exhibit and literature mentioned above were also presented in the Scientific Exhibits Section of the 38th Annual Conference of the American Physical Therapy Association held at Pittsburgh, Pennsylvania, June 26 - July 1, 1960. Miss Niebuhr monitored the Exhibit.

5. Again, the same Exhibit and literature were presented at the American Medical Association's Clinical Meeting, November 28 - December 1, 1960, Washington, D. C., with various staff members in attendance throughout this period.

6. On April 28, 1960 Miss Edith Maeda, OTR, was given an Award for Superior Performance at the Annual Awards Program of the National Institutes of Health.

SPACE:

The anticipated loss of one module in Occupational Therapy Service (4th floor, D-wing) and one module in Physical Therapy Service (5th floor, D-wing) because of requirements in connection with the new surgical wing occurred during 1960. The necessary relocation and reconstruction is proceeding satisfactorily and should be completed during the first half of 1961.

EQUIPMENT:

No major purchases of new equipment were made during the year.

PERSONNEL CHANGES:

A number of personnel changes took place in the Physical Therapy and Occupational Therapy Services with 3 resignations and 4 replacements. At the close of calendar year 1960, personnel of the Department totaled 24, with one vacancy in the Physical Therapy Service, and one vacancy in the Occupational Therapy Service.

Calendar Year 1960

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

PHARMACY DEPARTMENT CC-42
Serial No.

The Pharmacy Department is comprised of the following services: Pharmacy, Central Sterile Supply, Pharmaceutical Development, Radiopharmaceutical, and the Office of the Chief. Activities in some areas of service have been expanded, and highly improved; however, in other areas it was difficult to maintain the same level of service. In the area of the Pharmaceutical Development Service it could not be maintained at the high level set in 1959. Details of each area of responsibility follow under the respective service:

PHARMACY SERVICE

The major effort toward improvement of overall service was to bring up-to-date a portion of the physical facilities to reflect changing trends in medical research and treatment needs. These needs are greatly reflected in changes in drug preparations in addition to the ever changing field of commercially available drugs and packaging techniques. After seven years of operation, the time had arrived to alter some physical facilities, within limitations, in order to increase efficiency to the previously planned levels and to utilize space more fully.

The prescription dispensing area was redesigned and cabinets were relocated within the same general area but it resulted in a more compact unit within less space and considerably raised the daily output of prescriptions per man hour. In addition, it made available an ophthalmic storage area as well as a narcotic issuing and record keeping area.

The Unit Issue area was completely redesigned and rearranged. Two work counter units were removed and replaced with seventeen shelving sections. This provided storage and issue space for a greater variety of prepackaged items as listed in the Department Catalogue. Further, it provided the ideal opportunity for pre-printed Pharmacy Service requisitions for the most frequently issued items. These requisitions reduce professional nursing time in ordering supplies and greatly speeds the filling time within the Pharmacy.

All glass gallon bottles have been replaced with high density polyethylene plastic containers. This reduced the hazard of breaking containers of volatile or staining substances in the Pharmacy, during dumbwaiter delivery and while in use within the nursing unit. By reducing space needs for each container, the Service has been able to increase prepackaged gallon containers by at least ten percent and store them in the same amount of space.

Discharge and follow-up medication labels contain the printed statement "KEEP OUT OF REACH OF CHILDREN" as was strongly recommended by the Surgeon General.

Pharmacists within the Service have been rotated through all areas so as to train personnel in the total operation for increased efficiency.

Dispersing of investigational drugs has increased considerably and has become an important part of the role of the Clinical Center Pharmacy Department.

The prescription workload has increased 4.5% over fiscal 1959, whereas the other items of issue have increased by approximately 11%.

CENTRAL STERILE SUPPLY SERVICE

The Central Sterile Supply Service issues increased by approximately 16½% during the last fiscal year. Again there was no increase in personnel to off-set this increase. The increase was absorbed through several mechanisms. One is the revision of the preprinted sterile supply requisition to make it more effective, and in addition a new preprinted requisition for the surgery service items was placed in use. The Department, and particularly persons working on the Service, is continuing to evaluate new types of packaging to arrive at more efficient methods. This has resulted in the use of a new type of wrapping paper cut to size, which has reduced requirements for this operation.

Packaging and product research has continued as one of the big functions of the supervisory personnel of the Central Sterile Supply Service during the past year. It has resulted in the use of a higher percentage of disposable plastic syringes by obtaining the very best quality available; a special note of interest is that it is not the most expensive brand. For use with radioactive materials, it has eliminated, to a great extent, a waste of glass syringes. In most instances it has proved to be more economical than a 2 cc. glass syringe. The effort to have more plastic syringes used will be continued. We are not in a position to force the professional staff to use these; however, it should be encouraged more strongly in the future.

The largest increase in the Sterile Supply Service has been within the area of the Surgery Packaging Unit. This was one of the problems within the Service. There was a 56% increase through October, 1960 over the equivalent period of 1959. Several personnel have had to be reassigned to handle this increase. It has speeded up the effort to search out additional disposable items to offset the terrific demand upon personnel at a time when reassignment was very difficult.

One of the largest contributions has been in providing specialized items to the clinicians. It is felt that this is one of the most significant contributions made.

During this year there was placed on order a 24"-36"-60" steam and ethylene oxide gas combination sterilizer, which should be delivered in the early months of 1961. This will replace a larger and inefficient steam autoclave

with one having a dual function, which will relieve any autoclaving bottleneck and make the sterilizing services more complete since this will provide a larger choice of sterilizing agents. There has been a small ethylene oxide sterilizer available, which is not suitable for all plastics, since the freon diluent attacks some of the plastics. This will be eliminated, to a great extent, with the carbon dioxide-ethylene oxide mixture used in the new sterilizer.

As new plastic items are becoming more available for use, the question of toxicity of plastics is becoming much more important. There have been some toxicities reported in the food industry with several plastics, and there is considerably more thought given to possible toxicity of certain disposable plastic tubes which are used intravenously and within other body cavaties. The question of polyvinyl chloride sheeting toxicity for use during neurosurgery arose during the year. This has resulted in several consultations with plastic experts and undoubtedly will necessitate more in the future. Industry usually is not in a position to toxicity-test some of these items for the unusual use to which they may be adapted within the Clinical Center. Several professional personnel have stated that if items are approved by the Food and Drug Administration for food packaging, they certainly should be alright for disposable items.

This is not necessarily true, since the Food and Drug Administration approval for food packaging is usually for oral ingestion while many of the patient items are for intravenous use. These problems will need extensive consideration during the coming year and the Office of the Chief of the Pharmacy Department is prepared to carry on with this perplexing problem of toxicity to keep the patient items safe.

Space for the proper expansion of service within the Central Sterile Supply Service has become even more critical than was reported during 1959. The tremendous increase in the total number of items issued has taken its toll upon the space available. It was essential that the surgery packaging area be immediately increased and this resulted in the loss of space to other areas. The present temporary arrangement, awaiting the assignment of additional space, is very unsatisfactory; however, it is the lesser of two evils in that it does provide the surgery packaging area the space it absolutely needs. It is hoped that the Blood Bank area adjacent to the Central Sterile Supply room will be available for expansion of services for the entire Pharmacy Department early in the 1961 calendar year. Packaging research has been conducted with individual packages of alcohol sponges. These show great possibility for the coming year and will be followed by the staff on an extensive basis.

The introduction on a trial basis of a sterile disposable catherization tray will take considerable cost study and evaluation.

PHARMACEUTICAL DEVELOPMENT SERVICE

The total work load for the Pharmaceutical Development Service has increased slightly over the preceeding year. This has been done with great difficulty. The number of units has increased, mainly due to increased batch sizes of the investigational drugs registered. The loss of the Chief of this Service

early in the year has affected the effectiveness and total service rendered. Early in the year it became apparent that we would not be able to recruit a qualified person for this service. It became necessary to assign Mr. William Briner as acting Chief for this Service, in addition to his primary duty as Chief of the Radiopharmaceutical Service. This has not solved the personnel problem, but it has somewhat eased the Administrative responsibility within the Department. There has been a lessening of the effectiveness and leadership of this Service, since there has not been available the expert and close supervision necessary for the maintenance of a high quality service. It has continued to offer service but has not grown with the rest of the Department. The possibility of hiring two pharmacists with Doctor of Philosophy Degrees has been seriously investigated, but neither one was available at the salary which could be offered. There was one other applicant for the Service and it was felt that he was unsuitable. He was willing to take a much lower salary but had not completed his Ph.D. degree and probably never would if he found a satisfactory position. This personnel problem must be resolved soon or adjustments in services and functions must be made.

Lack of staff during the past year has limited this Service to routine duties with no time available for new product developments.

Again an urgent need is reported for suitable space and equipment for certain activities within the Pharmaceutical Development Service. As was mentioned in the 1959 report, a separate room or cubicle is becoming ever more necessary for preparing potent pharmaceutical products which have not had their toxicity established. During 1960 two Pharmacy Department personnel were affected by a new drug while working within the chemical hood. This has now been redesigned and rebuilt. It was found to be faulty; however, this only points up the fact that this is becoming an increasing problem. Once personnel have been affected by some of these drugs, there may be other products which will show toxic symptoms and which can build up within the area. An isolated room under negative pressure is badly needed for this activity before more, and perhaps graver, personnel injuries result.

RADIOPHARMACEUTICAL SERVICE

The work load statistics show an increase in the Radiopharmaceutical Service; however, the total increase in service is not revealed by these figures. While the total number of units formulated has not increased tremendously, the complexity of types of products formulated in 1960 is considerably greater than in 1959. Not shown in the work load data is the average professional time expended each month which is not directly related to the formulation of a given end product. This may be called non-productive or planning time as far as completed work units are concerned. However, it is one of the essential parts of the Service which eventually leads to a completed product. In 1959 the average monthly expenditure of this so-called planning time was 18 hours, while in 1960 this average increased to approximately 40 hours each month. This time is consumed in consultations with NIH clinicians, visitors to NIH and Library research. Radiation surveys of the Service Laboratory Area to detect contamination are included in this catagory. The importance of this service has grown during the year to a point where a second pharmacist was

assigned to it during the month of December, 1960. There is every indication that it will continue to grow in importance to the Clinical area.

Following are a few major contributions provided to the patient care area of the Clinical Center, to the National Institutes of Health, to the Public Health Service, and to the community.

In cooperation with the General Medicine Branch of the National Cancer Institute, the Service labeled Human Serum Albumin with Radioactive Chrominum-51. The end product in the production of Krypton-85 Injection for the Surgery Branch of the National Heart Institute proved to be much superior as compared to an earlier formulation.

During August, 1960 Walter Reed Army Medical Center and the Pharmacy Department of the Clinical Center entered into a temporary agreement involving the occasional production of a patient radiopharmaceutical product. This involves the preparation of products until such a time as Walter Reed Army Medical Center establishes a facility of its own. One of the first products prepared for them was DL-NEVALONIC ACID-1-C-14 Injection. Since then several other products have been prepared.

The Krypton-85 Injection preparation which was successfully developed by the Radiopharmaceutical Service has resulted in numerous visitors for orientation concerning the preparation of the product, or assistance concerning some phase of the preparation. Visitors to the Service included Dr. Albertie Benchimol and Dr. Ernest Crow of the University of Kansas Medical School; the Chief of the Nuclear Medicine Section of the Brooks Air Force Base Hospital, San Antonio, Texas; a professor of the University of Arkansas Medical Center, Little Rock, Arkansas; a physician in the Cardiology Section of the University of Minnesota, School of Medicine; a professor of the State University of New York in New York City, and physicians from Chicago, Brooklyn and other areas. These persons have been oriented in the procedure used for the preparation of Krypton-85 since it is not commercially available. Numerous visitors from industry have visited the Service. Several of these visits have concerned apparent discrepencies in completed products which industry is attempting to make after they have been developed and produced within the Radiopharmaceutical Service. After expending months of research, the directors of quality control of the E. R. Squibb & Sons and the Abbott Laboratories each consulted with Mr. Briner concerning their efforts in obtaining several suitable products for serving many requests which cannot be supplied from the Clinical Center. It is felt that this is an important contribution by the Radiopharmaceutical Service. The Department frequently consults with industry to obtain information which will benefit the preparation or development of a suitable product for the Clinical Center. It has become very apparent to the Chief of the Pharmacy Department that the two major pharmaceutical houses producing radiopharmaceuticals do not have all the developmental know-how.

Mr. Briner, Chief of the Radiopharmaceutical Service has participated in at least three closed circuit television presentations concerning radiation and its use for treatment of patients within the Clinical Center. A radiobiologist of the radiological health training activities at the Robt. A. Taft Sanitary Engineering Center consulted this Service concerning biological

experiments with radio isotopes which will be included in training courses at the Taft Center. The Chief of the Service has participated in the orientation of numerous nursing groups, the pharmacy of the Bureau of Medical Services, Division of Hospitals and other visitors who have spent varying times in the Clinical Center.

The major problems of the Department are involved with space and personnel. When more space is made available there will be an adjustment of space allocations made within the various service areas. One of the most critical is the Radiopharmaceutical Service, since it has experienced the largest increase in its operation. It is now overcrowded, and therefore suitable space was not available for the completion of several products. Also, its very small office area is immediately within the laboratory preparation area. This exposes all personnel to an unnecessary radiation hazard. This, it is hoped, can be corrected in the near future.

Space for the Central Sterile Supply Service is critical. The temporary relocation of physical facilities was essential in order to allow an increase within the Surgery Packaging area. This area will need to be increased further when the new surgical wing is opened. In addition, an adjustment in space and the relocation of many areas will need to be made. The Pharmacy Service and Pharmaceutical Development Service need additional space in certain areas of operation.

There exists an immediate need for an area in which very toxic materials, or materials of unknown toxicity, can be processed with more safety.

There is need to increase the number of aseptic transfer hoods to two in the Department.

The Department is in dire need of an overall quality control service as a portion of the Office of the Chief. Space and personnel have not been available. This facility must be provided with space and personnel as an essential portion of the overall responsibility for first line quality service. The Department provides good service; however, we are not in a position to carry on quality controls. This is providing an "almost excellent" service, but we are not sure of the quality and potency of some of the end products. This is particularly important within the Pharmaceutical Development Service area. More information concerning this quality control can be found within the Pharmacy Department survey.

Packaging space is a very pressing need for the Pharmacy Service in order to allow it to be accomplished more efficiently. As was mentioned in the 1959 report, it is hoped that the Blood Bank area can be assigned to the Pharmacy Department. Use of this area, together with adjustment and relocation of several existing facilities, will enable the Department to operate more efficiently.

A tremendous problem remains in the area of properly trained personnel. It has become common that, once personnel are trained, they move to more promising positions within the Public Health Service or resign. The position of Chief of the Pharmaceutical Development Service has been vacant for the period of one year. Several suitable applicants were interviewed but the Clinical

Center could not meet, or even come close to meeting, their salary requirements. This is partly due to the fact that the Pharmacy officer catagory is restricted on promotions while some of the other catagories, such as scientists, are not. Therefore, why should a Ph.D. with a background in Pharmacy apply as a pharmacist? He can receive unrestricted promotions if he can qualify as a scientist. It is hoped that this acute problem can be overcome during the early months of 1961, or it may become necessary to re-evaluate the Department's ability to carry on the Pharmaceutical Development Service functions without the proper personnel available.

When personnel are properly trained within the Department, the pharmaceutical industry frequently lures qualified individuals away by higher salaries. There was a time when the Service had better working conditions, better retirement, and better medical benefits to offer our employees, particularly in the commissioned corps. This is no longer true, since industry now is offering a much higher salary with fringe benefits equal, or superior to, those of the commissioned corps.

PHARMACY DEPARTMENT STATISTICAL DATA

	1957 (Calender)	1958 (Calender)	1958 (Fiscal)	1959 (Fiscal)	1960 (Fiscal)	1960 (Calender)
Total number of units issued	2,457,422	3,066,939	2,872,022	3,110,519	3,613,102	3,768,950
Pharmacy Service						
Prescriptions	16,034	20,436	17,453	23,775	24,907	24,989
Other Items	197,291	208,313	206,440	218,484	244,010	249,226
Total	213,325	228,749	223,893	242,259	268,917	274,215
Central Sterile Supply Service						
Units Issued	2,228,207	2,823,340	2,638,179	2,848,488	3,320,222	3,457,763
Pharmaceutical Development Service						
Units Developed and Issued	15,890	14,081	9,366	18,587	21,912	34,983
Requests Processed	1,210	678	992	784	923	1,010
Investigational Drugs Registered	---	---	---	488	339	302
Radiopharmaceutical Service						
Units Developed and Issues	---	769	584	1,185	2,051	1,989
Requests Processed	---	127	107	135	303	357
Pharmacy Officer of the Day						
Number of Requests for Service	---	---	---	---		910

PUBLICATIONS, SPEECHES GIVEN, OR SIGNIFICANT ACCOMPLISHMENTS BY PHARMACEUTICAL DEPARTMENT PERSONNEL INCLUDE

MILTON W. SKOLAUT

Contributing editor to the AMERICAN JOURNAL OF HOSPITAL PHARMACY, articles entitled "Pharmacy - Central Sterile Supply Services" in Vol. 17, November 1960, Vol. 17, June 1960, and Vol. 17, April 1960.

Narrated a one-hour closed circuit television presentation of the "Pharmacy Department Techniques and Precedures" to the American Pharmaceutical Association at the Shoreham Hotel, August, 1960.

"Equipment and Techniques for Sterile Dispensing of Ophthalmic Solutions" co-authored with Philip R. Hugill and Boris J. Osheroff - AMERICAN JOURNAL OF HOSPITAL PHARMACY, Vol. 17, September 1960.

"Suggested Criteria for Proper Labeling of Solutions Requiring Reconstitution" co-authored with Philip R. Hugill - AMERICAN JOURNAL OF HOSPITAL PHARMACY, Vol. 17, June 1960.

"A New Membrane Filter for Precision Filtration" co-authored with William H. Briner - JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION, Practical Pharmacy Edition, Vol. 21, May, 1960.

WILLIAM H. BRINER

Radiopharmaceuticals Are Drugs" - MODERN HOSPITALS, August, 1960.

"Nuclear Medicine Has Come of Age" - AMERICAN JOURNAL OF HOSPITAL PHARMACY, Vol. 17, June 1960.

"A New Membrane Filter for Precision Filtration" co-authored with Milton W. Skolaut - JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION, Practical Pharmacy Edition, Vol. 21, May, 1960.

Member of Orientation Course on closed circuit television program, once for the Compass Course of NIH and twice for the Division of Personnel.

Numerous speeches presented to Nursing groups and Professional Pharmacy groups throughout the year.

Received the CERTIFICATE OF HONOR presented by the General Alumni Association of Temple University for the most outstanding contribution to society through advancement in his field of Pharmacy.

PHILIP R. HUGILL

"Equipment and Techniques for Sterile Dispensing of Ophthalmic Solutions" co-authored with Boris J. Osheroff and Milton W. Skolaut - AMERICAN JOURNAL OF HOSPITAL PHARMACY, Vol. 17, September, 1960.

"Suggested Criteria for Proper Labeling of Solutions Requiring Reconstitution" co-authored with Milton W. Skolaut - AMERICAN JOURNAL OF HOSPITAL PHARMACY, Vol. 17, June, 1960.

"Polyvinyl Alcohol Packaging" - AMERICAN JOURNAL OF HOSPITAL PHARMACY, Vol. 17, October, 1960.

Presentation to the American Society of Hospital Pharmacists, August, 1960, entitled "Polyvinyl Alcohol Packaging" in hospital pharmacy.

BORIS J. OSHEROFF

"Equipment and Techniques for Sterile Dispensing of Ophthalmic Solutions" co-authored with Philip R. Hugill and Milton W. Skolaut - AMERICAN JOURNAL OF HOSPITAL PHARMACY, Vol. 17, September, 1960.

"Development and Clinical Investigation of a New Oral Surface Anesthetic for Acute and Chronic Oral Lesions" co-authored with Irwin I. Shipp, D.M.D., and Anderson F. Williams, D.M.D. - ORAL SURGERY, ORAL MEDICINE AND ORAL PATHOLOGY, Vol. 13, No. 5, May, 1960

CARL H. BROWN

Vice-Chairman, Section on Military Pharmacy, American Pharmaceutical Association.

Calendar Year 1960

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

MEDICAL RECORD DEPARTMENT CC-46
Serial No.

OBJECTIVES:

The Medical Record Department is delegated the major responsibility of insuring the completeness, accuracy, safekeeping and availability of medical records by maintaining the following internal controls: (1) Continual appraisal of the medical records of patients registered at the Clinical Center, in order to maintain the standards established by the Clinical Center and accrediting agencies; (2) Maintenance of required indexes; namely, the analysis of discharged patients and the diseases and operations index (based on Standard Nomenclature of Diseases and Operations), with a view toward providing adequate basic data to physicians participating in Medical research; (3) Maintenance, compilation and distribution of statistical data, concerning patient care, for utilization by administrative, clinical and research directors; (4) Maintenance of a systematic arrangement of medical records to insure their immediate accessibility for purposes of patient care service and medical research; (5) Maintenance of a system whereby the quantity and quality of dictated medical record reports are controlled; (6) Maintenance of an adequate control system with regard to the release of medical reports, medical information and medical records to authorized recipients; (7) Maintenance of a perpetual patient's index (Soundex) and patient registry.

PROGRESS MADE:

During the year 1960 there were 3,658 medical records received for qualitative and quantitative analyses and posted to the discharge analysis; and 6,522 medical records coded and indexed. Also during the year, 96 research projects, using groups of medical records, were accomplished, and 47 special statistical studies were completed.

To execute basic controls properly, standard operative procedures were supported and new ones developed as follows:

Education and Training:-

A handbook, which outlines the services provided and regulations enforced by the Medical Record Department, was developed and published for distribution primarily to physicians participating in patient care and is available to visitors and other key personnel at the Clinical Center. The handbook offers general orientation regarding the objectives concerning medical record keeping.

A handbook on Standard Nomenclature of Diseases and Operations was purchased for distribution to physicians participating in patient care.

The booklet consists of basic prinicples of terminology and coding of diagnostic entities in order to facilitate the adaptation of numbered codes to established diagnoses.

A procedure of orientation was established to include all employees of the Medical Record Department. The orientation consists of four sessions of lecture and question and answer periods; supervisors conduct the sessions related to their respective sections. One session includes general information concerning the organization of and services provided by Clinical Center departments and Institutes.

Orientation lecture tours continue to be given to personnel of the Nursing Department, Social Service Department, United States Public Health Service graduate medical record librarian students and other visitors to the Clinical Center.

Supervised training of two clerks in medical terminology and the basic principles of coding and indexing in accordance with Standard Nomenclature of Diseases and Operations; supervised review of medical terminology conducted by the supervisor with the medical transcribers.

Approval of leave without pay was granted one employee for the purpose of receiving formal training at the school for medical record librarians at the United States Public Health Service Hospital in Baltimore; the period of training is for one year which began on September 12, 1960. Upon completion of the course, said employee will be delegated supervisory responsibilities within the department.

Publications:-

Burich, G. S. and Tracy, E. P.; "The Use of Medical Records in Research"; JOURNAL OF THE AMERICAN ASSOCIATION OF MEDICAL RECORD LIBRARIANS; Volume 31, No. 4, pps. 143-144-169; August 1960.

Personnel:-

One qualified medical record librarian resigned and one was employed as Acting Chief of the Files Section; two key assistant supervisory positions are vacant. There are about ten positions in the department requiring an audit by a Position Classifier.

Procedures Involving Institutes:-

New procedures were established with National Cancer Institute and National Institute of Dental Research, with a view toward facilitating the processing of medical reports and medical records more conveniently and promptly by physicians.

A new procedure was established with National Institute of Mental Health regarding registration and medical record requirements for patients

admitted for psychological testing.

Procedures Involving Medical Record Department:-

Due to improved conditions resulting from the physical rearrangement of furniture, acquiring of minimal additional space and an adequate staff, all sections in the department have maintained their current workload. In addition, the staff has been able to decrease backlogs which had been in existence since the early years of operation.

The required indexes have been expanded to include an index of admitting diagnoses and an Admission Analysis Registry. The need for more detailed information and statistics based on admission resulted from an increasing number of requests from administrative, clinical and research directors.

The tentative schedule established to provide Sunday tour of duty by personnel of the Files Section has been discontinued.

The Index of Incomplete Records established in 1959, resulted in a decreased number of incomplete records and the lowest number of incomplete records since 1953. The index consists of a listing of incomplete records or deficiencies by individual case, type of deficiency, responsible physician and Institute. There follows a summary of incomplete records by month of discharge and Institute:

	NCI	NHI	NIAID	NIAMD	NIDR	NIMH	NINDB	Total
January			1					1
February								-
March								-
April				2	1	1		4
May			5	2				7
June			1	2				3
July	1	1		7	1	1	2	13
August	2	3	3	7	2	2	2	21
September	4	6	8	12	2	4	1	37
October	6	11	6	6	6	2	1	38
November	23	27	5	15	3	6	9	88
December	69	88	25	44	28	25	69	348
TOTAL a/o 1-16-61	105	136	54	97	43	41	84	560

Medical Record Department

Work Production for the department is summarized below as related to respective sections.

Medicolegal Unit received, screened, completed or prepared for mailing purposes 3,957 pieces of material such as medical reports, letters, insurance forms and other related material.

Medical Record Files Section: A total of 29,253 masters were reproduced during the calendar year consisting of 253 actual working days and 65 additional days or 520 hours of overtime; there was an increase of 21.3% over work received and produced during calendar year 1959.

A total of 18,890 medical records were pulled and delivered for purposes of research study or review for completion; 24,290 records were pulled and delivered for inpatient admissions and outpatient visits.

A total of 4,654 pages were duplicated by verifaxing; these pages include medical reports, correspondence, insurance forms and other related material for Clinical Center departments and Institutes.

Medical Record Transcribing Unit:

	1959	1960	Percentage
Discs received	7,706	7,783	1% increase
Discs transcribed	8,249	7,710	6% decrease
Masters and reports, pages transcribed	27,362	28,220	3% increase
Remaining work	207	416	50% increase

The remaining work includes discs to be transcribed, reports to be reviewed, signed or duplicated. Of the reports sent to physicians for signature and not returned, the earliest date of receipt of disc is 11-18-60; reports not yet transcribed, the earliest date of receipt of disc is 12-16-60.

The daily average number of transcribers was 22; daily average number of pages transcribed was 90. The work was completed during 253 actual work days and 61 additional days or 486½ overtime hours.

MAJOR PROBLEMS:

Backlog of coding and indexing of medical records; records for period from July 1953 through July 31, 1958 and from January through July 31, 1959 have been completed. A total of 5,416 records are outstanding, that is, remaining to be coded.

Unsuccessful recruitment of qualified medical record librarians.

Retaining operators trained in the use of the offset duplicating machine (Multigraph); the major reason for leaving is better pay. As a result of the turnover, a continuous problem exists; that is, we have inexperienced operators which results in backlog of work and delays the release of medical reports, frequent breakdown of machine and an increase in overtime.

PROPOSED FUTURE OBJECTIVES:

Keep abreast of the current workload.

Review existing procedures in which this department is involved and which relate to other departments and Institutes and as necessary, recommend revisions.

Medical Record Department

STATISTICS - Calendar Year 1960

I. Registration

A. Patients			5831
Inpatient		3668	
First Registration	1720		
Readmission	1948		
Outpatient (First Registration)		2904	
Walk-In		52	
Emergency		17	
B. Carded for Record Only			4
C. Employee Registration			1810

II. Patient Care Statistics

Total Number of Admissions	3,668
Total Number of Discharges (includes deaths)	3,658
Total Patient Days	139,246
Available Bed Days	188,856
Daily Average Number of Patients	380
Average Length of Stay	37
Percentage of Bed Occupancy	74
Total Number of Deaths	237
Total Number of Autopsies	222
Total Number of Patients admitted from July 6, 1953 to December 31, 1960	19,462
Percentage of patients admitted during 1960 came from Maryland, Virginia and the District of Columbia	51.5%

III. Geographic Distribution of Inpatients Discharged from the Clinical Center (3,658) and from Institutes by Transfers (109); Calendar Year 1960.

REGION	NCI	NHI	NIAID	NIAMD	NIDR	NIMH	NINDB	TOTAL
New England	10	23	7	11	-	4	24	79
Middle Atlantic	112	217	74	69	3	54	77	606
East North Central	55	59	8	32	4	17	25	200
West North Central	19	10	2	7	1	12	8	59
South Atlantic	637	645	262	304	37	42	553	2480
East South Central	28	69	14	20	-	4	23	158
West South Central	19	15	4	3	-	2	12	55
Mountain	4	10	1	5	-	2	7	29
Pacific	10	24	4	3	-	4	17	62
U. S. Territories	-	1	2	-	-	-	2	5
OTHER, Non-U.S.	6	16	3	4	-	-	5	34
TOTAL	900	1089	381	458	45	141	753	3767
a/ Specific Region								
District of Columbia	116	131	41	68	8	12	145	521
Maryland	193	204	86	90	20	15	192	800
Virginia	148	153	60	67	4	11	126	569
TOTAL	457	488	187	225	32	38	463	1890

a/ These figures are included under general region, South Atlantic.

IV. Specific Geographic Distribution of Inpatients Discharged from the Clinical Center by Calendar Year

	Patients Discharged					Percentage				
Calendar Year SPECIFIC AREA	1956	1957	1958	1959	1960	1956	1957	1958	1959	1960
District of Columbia	551	610	602	560	522	23	21	18	16	14
Maryland	589	799	810	760	797	25	28	25	22	22
Virginia	377	478	442	598	564	16	17	13	17	15
TOTALS	1517	1887	1854	1918	1883	64	66	57	55	51.5

V. Inpatients Discharged from the Clinical Center by Calendar Year.

Calendar Year	1956	1957	1958	1959	1960
Number of Discharges	2369	2865	3275	3485	3658

Calendar Year 1960

PUBLIC HEALTH SERVICE, NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

NURSING DEPARTMENT

CC-51
Serial No.

A. Program Goals

The goals of the Nursing Department remain the same: (a) Provision of the best nursing care to patients possible with the staff available; (b) provision of assistance to the clinical investigators; (c) orientation, training, and supervision of personnel so that they may become more proficient in carrying out (a) and (b), and nursing research directed toward improving nursing administration and practice.

B. Developments and Trends

1. Recruitment of personnel. Although we still do not have the number of full-time professional staff nurses on duty we would like to have, we have reduced the number of vacancies during this past year. The quality of staff nurses seems to be improved, and we are attracting graduates of some of the better schools of nursing. During the past 2 years the accession of professional staff nurses has exceeded the separations by about 30 each year. This is encouraging as it seems to indicate that the turnover rate has been somewhat reduced.

2. The number of hours of continuous nursing care to patients was increased by approximately 3,000 hours over last year. This increase was reflected in the Heart, Cancer, Arthritis, and Metabolic Diseases Nursing Services.

3. The number of hours of nursing care provided by part-time nurses was increased by 15,771 over last year. Recruitment of part-time nurses has been maintained at a satisfactory level. At this writing, we have limited new appointments to nurses who can work on either the evening or night tours of duty, or on weekends, in order to equalize our pool of nurses for all three tours of duty and weekend work.

4. Although the overall daily average pediatric census has not increased this past year, there is an increase in the number of children under 6 years of age and the number of mentally retarded children. This has increased the work load on all nursing services except Psychiatric, as infants and small children may be found on nearly all nursing units of the other five clinical services. It looks

as though this trend is becoming more pronounced as the daily average number of children under 6 has been 45 to 55 percent of the total number of children under 14 years during the first 6 months of fiscal year 1961.

5. There has been an increasing number of research protocols on the clinical services which have demanded a substantial increase in nursing services both to patients and clinical investigators. Some examples follow: (a) Increasing number of children admitted for study on the Thyroid Physiology and Diseases Project; the Purine Metabolism and Biochemistry of Metabolic Diseases Project; the Cystic Fibrosis Project; and Mental Retardation Projects. Most of these children spend a great deal of time on a metabolic regime. (b) Blood platelet administration in leukemia, both to adults and children. (c) Metabolic studies on cancer have included patients who have far advanced disease, who are greatly debilitated, and who require a great deal of nursing care in addition to the time involved in carrying out the many and varied procedures associated with research. (d) The virus studies in which prisoner volunteers have been used. (e) The stress studies carried out on families of children who have leukemia.

C. Progress Made

1. Except for rare occasions, we have been able to meet the daily demands for nursing services adequately. This has been accomplished through cooperative planning and evaluation of needs from Service to Service and the cooperation of our staff members.

2. Our staff development program has been strengthened by the addition of a well qualified nurse officer whose assignment is to develop and carry out supervisory training for professional nurses at all levels. This nurse officer joined the staff on August 1, 1960, and has already begun to implement her plans, especially with supervisors, head nurses, and staff nurses GS-7.

3. A study of activities of nursing personnel, with Dr. Jane Wilcox as chief investigator, is underway. Most of the data will have been collected by January 1, 1961, after which the analysis of the data and preparation of the report will be completed. Through this study it is hoped to find whether there are areas in which utilization of staff could be more effective; areas in which it may be possible to improve quality of performance or nursing practice; areas in which training of personnel should be improved; and possibly areas in which realignment of functions and activities may be advantageous.

4. There are pediatricians on the Heart, Cancer, Arthritis and Metabolic Diseases, and Allergy and Infectious Diseases Nursing Services. This has stimulated the formulation of standards and policies for both the medical and nursing staffs and thus has improved the care of children.

5. Problems associated with the care of patients with emotional disturbances have been reduced since psychiatrists participate in the interdisciplinary conferences and also have given more consultative services. As a result of this participation, morale of the nursing and medical staffs has been improved. This has been particularly noticeable on the Cancer Nursing Service.

D. Major Problems

A number of the problems reported last year are still unsolved, although attention has been directed toward them, and efforts have been made to seek a solution by all concerned with them.

1. Projects are still being planned around children without adequate consideration of the space and other facilities needed. We still lack play space and recreational facilities. Children are admitted to nearly every unit (except on Psychiatry). Children are placed in the same room with adult patients much too frequently. Mentally retarded children who are destructive and difficult to manage, and who require a great deal of the time, attention, and energies of the staff are admitted to units where there are patients in other projects who also require much attention and time of the staff. There have been times when it has not been possible to isolate children adequately until such time as it could be determined whether they were infectious or susceptible to infections present on the unit. The problems associated with a desegregated pediatric service are numerous and are deserving of thoughtful and intensive scrutiny by all of the professional groups concerned until some constructive plans for their solution are worked out and implemented.

2. There seems to be an increasing number of aged patients admitted for study. This also creates problems which must be handled, as many are senile and often almost psychotic. The care of the aged patient is quite specialized and not particularly well understood by either the medical or nursing staffs. With an aging population, we must expect to admit an increasing number of patients over 70 years of age and that the necessary planning for them must be done jointly by all professional groups concerned.

3. Absenteeism of staff (particularly because of "sick leave") has been a real problem. This has been most pronounced in the male nursing assistant group. A great amount of time has been spent in dealing with this to no apparent avail. It is difficult to cope with when it is possible to purchase a medical certificate for $1 or $2, under the sick leave regulations as written and interpreted.

4. There is a serious lack of instruction and explanation of research protocols on the part of the investigators. Nursing staff frequently are in a state of confusion because of this lack, or because of conflicting information. Present communications, which are time consuming, should be improved between the professional groups in order for the nursing staff to function more effectively and accurately.

5. Lack of space for essential activities for both patients and personnel is still present. This includes office and conference space for personnel, recreational space for children, and space for storage of equipment used in patient care.

E. Accomplishments

1. Publications

(a) Fitzwater, Janet, "The Bacteriological Effect of Ultraviolet Light on a Surgical Instrument table." American Journal of Nursing, January 1961; also in Public Health Reports.

(b) Fitzwater, Janet, "The Selection of Instruments for Major Operations." Nursing Research, Vol. 9, No. 3, 1960.

(c) Fitzwater, Janet, "Combined Position for Pelvic Surgery." Submitted for publication in the American Journal of Nursing.

(d) Edwards, Elizabeth, "Psycho-physical Aspects of the Nursing Care of Mycosis Fungoides." Submitted for publication in the American Journal of Nursing.

(e) Crawley, H. Mildred, "Nursing Care of Patients with Myocardial Infarctions." Submitted for publication in the American Journal of Nursing.

(f) Garzoni, Mary, "Quilted Slip Covers for Bed Side Rails." In the Trading Post section of the American Journal of Nursing, November 1960.

2. Awards

(a) To Mrs. Mary Garzoni for her suggestion about quilted covers for bed sides.

(b) To Miss Isabelle DeBella for her suggestion as to a calibrated device for measuring urinary drainage.

(c) To Mrs. Mary Mitchell, Miss Mary Shea, and Miss Marie Altman (group award) for Sustained Superior Performance.

(d) To nursing staff on 2 West (group award) for Sustained Superior Performance.

3. Other

Miss Barbara Saltow, Head Nurse, Neurology Nursing Service, was appointed to the Nursing Advisory Committee of the National Cerebral Palsy Association.

F. Plans for 1961

1. Continuous effort to improve in the various areas of our program goals.

2. Study the organizational structure of the Nursing Department with a view toward improving it for more efficient operation.

NUMBER ACCESSIONS TO THE NURSING DEPARTMENT
BY POSITION AND GRADE EACH MONTH
FISCAL YEAR 1960

Month	GS-11 and above	Head Nurse GS-9	Staff Nurse GS-7	Staff Nurse GS-5 and 6	Clerical GS-3 and 4	Practical Nurse GS-3	Nursing Ass't GS-2	Total
	No.	No.	No.	No.	No.	No.	No.	No.
1959 July			1	8	4		1	14
August			1	12	2	4	2	21
September		2		24			9	35
October			1	19	4	1		25
November				2	2	13	5	22
December				3		5		8
1960 Jan.			1	6	3	5	4	19
February				9	2			11
March				5			1	6
April			2	3	2	2	1	10
May				2	1		7	10
June			3	8	2	3		16
Total		2	9	101	22	33	30	197

NUMBER SEPARATIONS FROM THE NURSING DEPARTMENT
BY POSITION AND GRADE EACH MONTH
FISCAL YEAR 1960

Month	GS-11 and above	Head Nurse GS-9	Staff Nurse GS-7	Staff Nurse GS-5 and 6	Clerical GS-3 and 4	Practical Nurse GS-3 and 4	Nursing Ass't GS-2 and 3	Total
	No.	No.	No.	No.	No.	No.	No.	No.
1959 July			3	8		1	2	14
August			2	13	4	1	3	23
September		1	2	16	2		5	26
October			2	6		1	2	11
November		1	2	6	1	1	3	14
December			2	6	1		3	12
1960 Jan.		1	2	7	4	1		15
February		1	2	8		1	3	15
March			3	2		2		7
April			1	3			4	8
May		1	1	6	1		1	10
June			2	3	5		1	11
Total		5	24	84	18	8	27	166

NUMBER HOURS OF CONTINUOUS NURSING CARE
FISCAL YEAR 1960

Month	Nursing Service						
	Allergy and Infectious Diseases	Arthritis and Metabolic Diseases	Cancer	Heart	Neurology and Blindness	Psychiatric	Total
	Hours	Hours	Hours	Hours	Hours	Hours	Hours
1959 July	647	540	708	2,957	856	30	5,738
August	926	188	1,205	1,458 3/4	300	0	4,077 3/4
September	420	93	832 1/2	2,846 1/2	0	13	4,205
October	36	317	988 1/2	4,475 1/2	979 1/2	0	6,796 1/2
November	239	65	1,138	3,610	365	0	5,417
December	266	562	1,116	3,054	511	83 1/2	5,592 1/2
Jan. 1960	566	505 1/2	1,162	2,335	936	813 1/2	6,318
February	593	190	1,296 1/4	2,247	535	10 1/4	4,871 1/2
March	336	144	1,391 3/4	2,615	904	113 1/2	5,504 1/4
April	607	558	919 3/4	1,867	802	204	4,957 3/4
May	527	353	949	2,620	984	0	5,433
June	126 1/2	164	1,068	1,934	370	31	3,693 1/2
Total	5,289 1/2	3,679 1/2	12,774 3/4	32,019 3/4	7,542 /2	1,298 3/4	62,604 3/4 1/

1/ Equivalent to: 7,825.8 8-hour days
1,565.1 40-hour weeks
35.4 Man years

NUMBER HOURS OF OVERTIME
FISCAL YEAR 1960

Month	Nursing Service								
	Admissions and Follow-up	Allergy and Infectious Diseases	Arthritis and Metabolic Diseases	Cancer	Heart	Neurology and Blindness	Psychiatric	Surgery	Total
	Hours	Hours	Hours	Hours	Hours	Hours	Hours	Hours	Hours
1959 July	20 1/2	90	144	48 1/2	36 1/2	40	116 1/2	41 1/2	537 1/2
August	54 1/2	238	216 1/2	109	109	32	80 1/2	117 1/2	957
September	46 3/4	137 1/2	154 1/2	160	205 1/2	0	44	101 1/2	849 3/4
October	8 1/2	16	147	140	107	8	118	105	649 1/2
November	23	10 1/2	116	233	46 1/2	0	167 1/2	0	596 1/2
December	30	0	87 1/2	87 3/4	23 1/2	0	111 1/2	53 1/2	393 3/4
1960 Jan.	25 1/4	57	207	173	41 3/4	73	168 1/2	71 1/2	817
February	38	32	107 3/4	252	33 1/2	23	66	37	589 1/4
March	37	12 1/2	98	193 1/2	81 1/2	15	51 1/2	108	597
April	27 1/4	18 1/2	193	94	17 3/4	8	103 1/2	62 3/4	524 3/4
May	82 1/2	81	190	96	0	8	55 1/2	44	557
June	25 3/4	40	162 1/2	37 1/2	0	0	68 1/2	49 1/2	383 3/4
Total	419	733	1,823 3/4	1,624 1/4	702 1/2	207	1,151 1/2	791 3/4	7,452 3/4 1/

1/ Equivalent to: 931.6 8-hour days
186.3 40-hour weeks

NUMBER HOURS WORKED BY PART-TIME PROFESSIONAL
NURSING STAFF (W.A.E.) BY SERVICE
DURING FISCAL YEAR 1960

Month	Nursing Service							
	Allergy and Infectious Diseases	Arthritis and Metabolic Diseases	Cancer	Heart	Neurology and Blindness	Psychiatric	Surgery	Total
	Hours	Hours	Hours	Hours	Hours	Hours	Hours	Hours
1959 July	184	673 1/4	440	694 1/2	612	184	0	2,787 3/4
Aug.	280	561	517 1/2	400	485 1/2	240	0	2,484
Sept.	570	744	584 1/2	608 1/2	511	361	0	3,379
Oct.	223	736	1,077	783	521	336	0	3,676
Nov.	215	600	1,427 1/2	935	297	384	0	3,858 1/2
Dec.	138	620	1,528	911	256	231	0	3,684
1960 Jan.	227	696	1,085	860	412	271	0	3,551
Feb.	458	871	1,358	819	414	225 1/2	8	4,153 1/2
Mar.	202	584	1,790	1,242	474	320	32	4,644
Apr.	248 5/8	808 3/4	1,240 1/4	928 7/8	624 1/2	248	0	4,099
May	320	953 1/8	1,073 5/8	1,064	480 1/4	320	0	4,211
June	168	917	1,041 1/2	1,033	342	288	0	3,789 1/2
Total	3,233 5/8	8,764 1/8	13,162 7/8	10,278 7/8	5,429 1/4	3,408 1/2	40	44,317 1/4 1/

1/ Equivalent to: 5,539.6 8-hour days
1,107.9 40-hour weeks

DAILY AVERAGE CENSUS OF CHILDREN
EACH MONTH FISCAL YEAR 1960

Month	Under 2 years of age	2-5 years of age	6-13 years of age	14-16 years of age	Total
1959 July	3.3	14.1	36.0	18.3	71.3
Aug.	4.6	17.0	33.7	13.6	68.9
Sept.	2.4	21.7	28.8	10.9	63.8
Oct.	7.9	19.9	21.8	10.8	59.8
Nov.	5.3	18.0	25.5	9.5	58.3
Dec.	6.8	11.3	25.8	8.0	51.9
1960 Jan.	6.2	12.9	29.9	7.8	56.8
Feb.	5.9	13.3	35.8	12.7	67.6
Mar.	8.0	14.6	26.4	9.6	58.8
Apr.	9.2	14.1	28.7	8.3	60.2
May	7.9	15.4	24.9	2.9	51.6
June	8.3	17.9	35.1	3.5	64.8
Yearly average	6.3	15.8	29.3	9.7	61.2
Percent of total	10.3	25.8	47.9	15.8	100.0

DAILY AVERAGE CENSUS OF CHILDREN ON
EACH NURSING SERVICE EACH MONTH
DURING FISCAL YEAR 1960

Month	Nursing Service						
	Allergy and Infectious Diseases	Arthritis and Metabolic Diseases	Cancer	Heart	Neurology and Blindness	Psychiatric	Total
1959 July	8.9	13.6	19.2	17.9	11.7	1.0	71.3
Aug.	11.8	10.4	20.3	13.7	11.7	1.0	68.9
Sept.	10.9	9.0	19.3	16.1	7.4	1.0	63.8
Oct.	9.5	4.9	16.8	14.5	13.0	1.0	59.8
Nov.	6.5	4.5	17.5	18.6	9.9	1.0	58.3
Dec.	6.8	5.6	15.0	13.2	10.7	.7	51.9
1960 Jan.	7.6	9.9	17.1	10.0	11.5	.7	56.8
Feb.	10.9	8.0	18.2	12.4	16.4	.7	67.6
Mar.	10.9	8.3	13.5	11.8	14.4	.0	58.8
Apr.	9.3	9.3	16.3	10.7	14.9	.0	60.2
May	6.7	8.8	16.6	9.0	10.1	.0	51.6
June	5.8	12.5	18.2	13.7	14.9	.0	64.8
Average for year	8.8	8.7	17.3	13.5	12.2	.6	61.2
Percent of total	14.4	14.2	28.3	22.0	20.0	.9	100.0

NUMBER HOURS OF LEAVE TAKEN
DURING FISCAL YEAR 1960

	Profes-sional staff	Non-profes-sional staff	Clerical staff	Total staff
Daily average number personnel----------	299.1	245.4	44.0	588.5
Annual leave				
Total hours taken--------	36,611.0	27,202.0	4,736.0	68,549.0
Average hours per person-	122.4	110.8	107.6	116.5
Average working days per person----------	15.3	13.8	13.4	14.5
Sick leave				
Total hours taken--------	20,553.0	19,147.0	3,565.0	43,265.0
Average hours per person-	69.0	78.0	81.0	73.5
Average working days per person----------	8.6	9.7	10.1	9.2
Other leave				
Total hours taken--------	12,197.5	12,946.0	1,449.0	26,592.5
Average hours per person-	40.8	52.6	32.9	45.2
Average working days per person----------	5.1	6.6	4.1	5.7
Total hours of all leave	69,361.5	59,295.0	9,750.0	138,406.5
Average hours per person	231.8	241.6	221.5	235.2
Average working days per person----------	28.97	30.2	27.7	29.4

NUMBER OF PERSONNEL ON EXTENDED LWOP
STATUS DURING FISCAL YEAR 1960

Title and grade of position	On LWOP	Returned to duty	Resigned	Died	On LWOP 6/30/60
	No.	No.	No.	No.	No.
GS-9 Supervisor---------	2	0	1	0	1
GS-7 Staff Nurse--------	10	4	3	0	3
GS-5 and -6 Staff Nurse-	10	4	5	0	1
GS-3 and -4 Practical Nurse----------	17	10	3	0	4
GS-2 Nursing Assistant--	1	0	0	1	0
GS-4 Secretary----------	2	0	1	0	1
Total-------------------	42	18	13	1	10

PHS-NIH
Individual Project Report
Calendar Year 1960

Part A

Project Title:

A study of the Activities of Nursing Personnel on the Nursing Units of the Clinical Center, NIH.

Principal Investigator:

Dr. Jane Wilcox, Special Assistant for Nursing Research, Nursing Department, Clinical Center.

Other Investigators:

Research Assistant:	Miss Florence Seidler
Observers:	Miss Jean Brotslow
	Miss Alice Duncan
	Miss Florence Sato
	Mrs. Mary Thompson

Cooperating Units: None

Man Years (calendar year 1960):

Total:	1 2/3 man years
Professional:	1 1/2 man years
Other:	1/6 man year

Project Description:

a. Objectives:

(1) To obtain an overall picture of the activities in which nursing personnel at the Clinical Center are engaged.

(2) The information obtained may suggest the need for changes in content or emphasis in the administration of the nursing service on the nursing units. It may also indicate needs in the area of in-service education.

(3) Since the activities of nursing personnel in a clinical research center have not been studied in this manner before, this study provides an opportunity to determine

what adaptations in methods of studying nursing activities are necessary in a research institution.

b. Methods Employed:

(1) One unit each from the Allergy and Infectious Diseases, Arthritis and Metabolic Diseases, Cancer, Heart, and Neurology Nursing Services is being studied. This sample of five units includes a surgical unit, a metabolic unit, a communicable disease unit, and two medical units. Pediatric patients are regularly present on three of the units.

(2) The activities of nursing personnel were observed by "work sampling." This technique, first developed for use in industry, has been found to be well adapted to observing the type of non-repetitive activities performed by nursing personnel in hospitals. The technique was specifically modified and adapted to fit the situation at the Clinical Center.

(3) A classification of activities in Areas (patient centered, personnel centered, etc.) and Levels of Skill (professional nurse skills, clerical skills, etc.) was developed and tested in a pilot study on one unit. Specific categories to fit the situation at the Clinical Center were used.

(4) In the pilot study on one unit, attempts were made to develop a scheme whereby activities could be classified as to whether or not they were related to the conduct of clinical research. This did not prove fruitful and was not extended to the other units studied.

(5) The detailed descriptions of activities recorded on the observation forms are being searched for activities that might possibly be assigned to an administrative assistant.

c. Major Findings:

(1) From the method employed, it will be possible to derive estimates of the proportion of time nursing personnel spend in major categories of activities.

(2) Preliminary findings on the unit used for the pilot study confirm that the study will meet the objectives outlined above.

d. Significance:

(1) The findings will assist the Nursing Department

in evaluating the assignment and utilization of nursing personnel.

(2) The findings will enable the Nursing Department to determine whether nursing personnel are spending their time in activities which other persons could perform. For example, is there a need for an administrative assistant to the head nurse on the nursing units at the Clinical Center?

(3) The findings will assist the Nursing Department in determining and evaluating needs for in-service education of nursing personnel.

(4) The experience of this study will facilitate the further study of the nursing service program at the Clinical Center.

e. Proposed Course of Project:

(1) The collection of data will be completed early in January 1961.

(2) Machine tabulation of the data will be done by the Statistical Processing Unit, NIH.

(3) A report of the findings will be presented to the Chief, Nursing Department, and to the chiefs of the various nursing services involved as a basis for further action.

(4) Specific consideration will be given to the question of the desirability of trying the employment of an administrative assistant on a nursing unit.

Part B included No.

Calendar Year 1960

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

NUTRITION DEPARTMENT CC-61
Serial No.

I. CONTRIBUTIONS TO THE CLINICAL RESEARCH PROGRAM

The Nutrition Department's opportunity of contributing to the clinical research program has grown with the trend toward greater recognition of nutrition as a significant factor in medical research. Its contribution is made, for the most part, in its day to day patient food service operation, and secondly through special studies.

As of July 1, 1960, more than 46% of the protocols of the Clinical Center's current clinical studies included nutrition facets:

Percentage of Nutritional Implication in Current Clinical Studies

July, 1960

Institute	Number of Studies Listed*	Number of Studies with Nutritional Implications
NIAMD	10	10 (100%)
NHI	15	10 (67%)
NCI	24	11 (46%)
NIMH	5	2 (40%)
NIAID	18	7 (39%)
NINDB	10	3 (30%)
NIDR	10	0 (0%)
Totals	92	43 (46.7%)

*"Current Clinical Studies and Patient Referral Procedures, revised July 1960."

The Patient Dietetic Service staff conducted two studies and reported their findings in papers entitled "An Evaluation of Two Methods of Dietary Control, Based on Analyzed Data of Five Nutrients," and "Frequency of Meals and Total Caloric Intake."

II. MAJOR PROBLEMS ENCOUNTERED

Workload fluctuations, in the patient dietary service for all institutes as well as in the cafeteria operation, continue as the greatest problem, being extremely difficult to cope with because of the inflexible staffing pattern. For example, in October, 1960, Categories III and IV diet meals served to patients of NCI increased over September by approximately 1400 meals; in April,

for NHI, they increased over March by 1500. (By definition, Categories III and IV diets reflect the research need.) Cafeteria sales ranged, in 1959, from 63,292 a month to 74,956; and in 1960, from 65,492, to 77,944.

The patient ration cost, the economic barometer of the Department, has continued to rise as a result of (1) the wage-board personnel pay raise in January, 1960; (2) rising raw food costs; and, (3) incomplete utilization of metabolic kitchen facilities. Only 64% of metabolic kitchen facilities were utilized in fiscal year 1960.

Patient Ration Costs

Fiscal Year:	1958	1959	1960	1961 (thru 10/60)
Personnel :	$6.26 (77%)	$6.97 (80%)	$7.75 (81%)	$ 8.24 (81.5%)
Raw Food :	1.51	1.53	1.58	1.61
Sup.& Other:	.32	.25	.27	.26
Average cost per ration:	$8.09	$8.75	$9.60	$10.11

Personnel problems include the need for a new civil service examination to provide adequately qualified non-professional staff; difficulty in recruiting properly qualified clerical staff; professional staff turnover; and providing coverage for employees on sick leave.

The 11th Floor Kitchen faced a double problem in servicing NIAID's cold virus study--the necessity of using special isolation techniques and overloaded facilities, the patient census reaching as high as 58 and the kitchen being equipped to handle a maximum of 52.

Full isolation techniques had to be used for 7 months to protect leukemia patients on 2W from amebiasis developed by some of the 2W patients.

GAO began its audit of the Department in September, 1960. Many hours have been spent in conferences with the auditors.

Continuing problems are those of insufficient space, prevention of insect infestation, noise in the patient area, the need for constant maintenance of equipment and facilities, and finding sufficient time to assure many visitors pleasant and informative tours through the Department.

III. ACCOMPLISHMENTS, CHANGES, AND IMPROVEMENTS

From December 1, 1959, through November 30, 1960, the Nutrition Department provided 29,272 recorded daily dietary intakes (an average of more than 80 a day), 390,662 patients' meals, and weighed food on 263,448 individual trays. 255 diet aliquots (765 meals) were weighed and provided for laboratory analysis; 11,238 patient days of fluid intakes were measured; and 236 patients were given individual discharge or follow-up diet instructions, each with complete nutrition histories taken.

Twelve floor kitchens, a formula room, the Main Kitchen, and the 3-B (NCI) and 9th (NIAMD) metabolic kitchens were operated the full twelve months of the year. The 7th (NHI) metabolic kitchen was open 11 months and the 12th (NCI) less than 10 months.

The cafeteria, serving NIH personnel and visitors, was operated throughout the year. On February 20, 1960, a fast-moving check-out line was established for patrons desiring a limited number of items such as sandwiches and beverages.

Procedures were reviewed and improved. New ones were established as needed. Menu planning was changed for normal control patients on 4-West to better accommodate their preferences. Purchasing procedures were improved.

The Food Production Service staff worked throughout the year on a long range recipe standardization project in the interests of quality control. The adjustment on the books to reconcile accounts with the physical inventory amounted to less than $\frac{1}{2}\%$ of the total raw food cost in fiscal year 1960. A "Dietitian on Call" program became effective in January 1960.

In the field of personnel management, professional staff business meetings were held throughout the year; the semi-annual evaluation of non-professional employees, under promotion policy criteria, was completed and the policies reviewed with the staff; criteria for professional staff promotions were developed; the patient area staffing structure was reorganized; and under the Nutrition Department training program, non-professional supervisors were guided in setting up criteria for satisfactory job performance in positions they supervise.

Equipment and facilities in the kitchen areas were maintained on a continuing basis throughout the year. Items of new equipment were installed, some having been especially designed and fabricated.

Publications were: "Today's Needs in Food Service Equipment," by Edith A. Jones; "Frequency of Meals and Total Caloric Intake," by Ann Reimer and Barbara Dennis, Journal of the American Dietetic Association; "The Role of the Dietitian in the Research Program," by Edith A. Jones, Food and Nutrition News, National Live Stock and Meat Board.

IV. PROPOSED FUTURE OBJECTIVES

To evaluate the Department's dietary management techniques and the practice of restricting canned food items in control diets to one day's run at the cannery.

To revise patient diet instructions material used to supplement oral instructions to patients being discharged; the Food Portions Manual; the Nutrition Department diet calculation reference book; and specifications for perishable foods.

To prepare a manual explaining how to organize, equip, staff, and operate a metabolic kitchen, for distribution and eventual publication in connection with NIAMD's plans to sponsor metabolic kitchens in certain university hospitals; and to develop a film illustrating techniques used in metabolic kitchens here.

National Institutes of Health
Bethesda, Maryland 20014

DATE DUE

GAYLORD PRINTED IN U.S.A.

NIH LIBRARY
Amazing Research.
Amazing Help.

http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

NIH LIBRARY
4 0130 0419

NIH LIBRARY
3 1496 00198 0617